Enzyme and Metabolic Inhibitors

Volume III

Iodoacetate, Maleate, N-Ethylmaleimide, Alloxan, Quinones, Arsenicals
Volume I  General Principles of Enzyme Inhibition

Volume II  Malonate  
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            Dehydroacetate  
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            o-Iodosobenzoate  
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Volume IV  Uncouplers of Oxidative Phosphorylation
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            Naturally Occurring Inhibitors  
            Cholinesterase Inhibitors  
            Monoamine Oxidase Inhibitors  
            Drugs as Inhibitors  
            Carbonic Anhydrase Inhibitors  
            Borate
This volume is dedicated with sincere gratitude to the medical librarians — Vilma, Ruth, Lilian, Clara, Esther, Michelle, Rose, Shari, Nancy, Nahida, and others — who have not only helped me to the limit, but have made each visit to the library a pleasure and often the most delightful experience of the day.
For those rare readers who may feel inclined to pursue their way through Volumes II and III from beginning to end, I have tried to arrange the chapters and sections in a logical and interdependent order. Malonate has been approached first because its actions so well illustrate some of the general principles covered in Volume I, and, indeed, malonate is discussed in greater detail than any other inhibitor in order to suggest how one would like to deal with all inhibitors if one had either the time or space. Inasmuch as malonate is the classic substrate analog, the next chapter takes up various types of analogs and here we are able to obtain some rough idea of the energies involved in the interactions of inhibitors with enzyme surfaces, as well as study some of the factors which determine specificity. Some readers may feel that too much attention has been given to these analogs, but I believe they represent a very important group of inhibitors and illustrate many principles — competitive behavior, group specific interactions, protection and reversal, and even mutual depletion kinetics since some analogs are extremely potent inhibitors — and, in addition, contribute to our understanding of feedback inhibition and metabolic regulation. Most of the remainder of the volumes is devoted to substances considered to react with SH groups, certainly one of the most commonly used and important classes of inhibitors, about which it is surprisingly difficult to find adequate and comprehensive treatment. Certain aspects of inhibition have been treated in detail, not necessarily because of any intrinsic importance, but because of the information which is provided to help us comprehend the general phenomena of inhibition. There are many ways of writing about inhibitors, and I have tried to alter the approach according to what I believe to be the most interesting aspects of each inhibitor. These aspects may not happen to be those which would have been chosen by the reader, but it is impossible to cover any inhibitor completely and present it from all viewpoints. On the other hand, there are certain sections which I have been unable to make very interesting, to organize into a coherent picture, sometimes because the data are insufficient or too heterogeneous, but nevertheless some worthwhile material
can often be included in these areas. One finds much of the subject to be somewhat disconnected and it is seldom possible to present an orderly clear version of any inhibition because of the gaps in our knowledge, but one must consider that these isolated strands may some day be woven into a durable fabric. Each chapter has in general been organized so that the treatment proceeds from the simplest system to the higher levels of organization, since this generates progressive understanding it may be hoped, although one occasionally wishes that the effects on the simpler systems could be appreciated against a background of the actions on tissues and animals. Perhaps to some extent the historical introductions at the beginning of most chapters may serve as provisional backgrounds. As in many fields of science one is confronted with the problem of vertical or horizontal presentations. Efforts, however inadequate, have been made to correlate the results at the different levels, and it is hoped that unlike bacteria under certain conditions this volume does not too much exhibit the phenomenon of accumulation without synthesis, or suffer from an even worse danger, that in the psychosynthesis of concepts and over-all pictures some abnormal or spurious units have been lethally incorporated.

This peripus of the field of enzyme inhibition presents a rather large and often heterogeneous group of information, but everything has been selected for some reason; the reasons may be debatable, since different readers come to a book for different purposes, but occasionally one detects something in a report, perhaps intuitively, which others would not, and hence includes it for reasons difficult to express. The half-life for the general use of a book is, indeed, determined in part by the ability or good fortune of the author to select that which will have the most value or pertinence in the pseudopodal fronts of science. One has no time for justifications, since some decisions have to be made, and only the naive think they can please or help everyone, but there is perhaps one justification I feel impelled to make. Certainly there will be those who ask why the effects of an inhibitor on the blood pressure or the central nervous system have been presented when there is little or no obvious correlation with any metabolic inhibition, or why I have made up tables of tolerated or lethal doses, and, in general, some may criticize the discussion of inhibitor actions which are likely to be unrelated to enzyme inhibition, or at least for which there is no direct evidence. In defense of this, I can only say that I believe we should not so rigorously categorize the actions of inhibitors. The refusal to consider the nonmetabolic actions has led many investigators to very biased interpretations of their data. If we are interested in the mercurials, we are, I assume, interested in all their possible actions, whether they are
based on metabolic disturbances or not. To be narrow here would be like discussing only the beneficial effects of drugs and omitting the toxic actions. Of course, space limitations make it impossible to treat all these actions equally, and I have tried to emphasize those actions in which a disturbance of metabolism is the most likely mechanism. But we must never ignore the possibilities of other mechanisms with any inhibitor, particularly those reacting with groups on proteins and other cell components. The mercurials offer an especially clear example of enzyme inhibitors producing characteristic effects on many tissues (e.g. kidney, heart, central nervous system, liver, muscle, etc.) and where not a single action can be definitely correlated with a mechanism involving enzyme inhibition. Nevertheless, with further improvements in techniques and more knowledge, it is quite possible that in the future at least some of these actions will be related to effects on enzymes. To be perfectly honest, at the present time we cannot say in the majority of cases just how substances called enzyme inhibitors act to produce their interesting and often clinically or industrially important effects on microorganisms or tissues, and it is necessary to realize our ignorance so that progress in understanding may take place. Inhibitors do produce some very intriguing effects on tissue function or in whole animals, and many of these effects are unknown to those who look upon inhibitors merely as biochemical tools; so by reading of these effects some may be activated to study the ultimate causes in greater detail. Incidentally, since inhibitors will be used more and more frequently in animals, information on dosage ranges to produce various effects may serve a very practical purpose.

I would like to express my gratitude to those who have written to me saying they have found the first volume of interest or of some value to them, who have sent me unpublished manuscripts or difficultly obtainable material, and who have given me encouragement during those periods when I sincerely wished I were in a monastery in Kyoto.

J. Leyden Webb

November, 1965
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INTRODUCTION

Certain principles or prejudices in the approach should be clearly stated since these occasionally heretical opinions have been the basis for much of the organization of this volume.

What is hoped to be pertinent information on the physical and chemical properties of the inhibitors has been given in the belief that the proper use of any inhibitor requires as much knowledge of its properties as possible. Such data are often difficult to find and I am afraid that much important material has been omitted.

The quantitative formulation of inhibitions has been stressed because here, as in every science, progress often depends on accurate recording and reporting of observations. It is, for example, not only not informative but actually misleading to state that aldehyde oxidase is or is not inhibited by p-mercuribenzoate. What is meant by "inhibited" — 10%, 50%, or 100%? What is the concentration of the inhibitor? If it is 0.01 mM it may mean something, but if it is 10 mM probably nothing. What is the source of the enzyme? There are many aldehyde oxidases and they differ quite markedly according to their sources. What substrate and acceptor were used? Is the substrate acetaldehyde, glyceraldehyde, formaldehyde, retinene, or even hypoxanthine, and are electron acceptor dyes used or the O₂ uptake measured? These and other factors must be made explicit. Of course, it is impossible in a book like this to give a complete picture of each inhibition mentioned, but I have tried to state the source of the preparation, the substrate, the inhibitor concentration, and the per cent inhibition in every case, as well as the pH and the incubation times when necessary.

One can understand a phenomenon better when it can be visualized in some manner and much of the recent work on the inhibition of enzymes has been done with the purpose of clarifying the topography of the enzyme surface and the nature of the interactions occurring there. Thus I have tried to emphasize the interpretation of data in terms of an accurate delineation of group orientation and intermolecular forces, although in the present state of our knowledge this can seldom be done satisfactorily.
Metabolism within cells is almost always a matter of multienzyme systems and so the effects of inhibitors on such systems have been discussed fully wherever possible, although this is even more difficult to describe quantitatively than the behavior of single enzymes.

The importance of the specificity of inhibition was sufficiently emphasized in the previous volume and it should be clear that this is a critical problem which has been neglected, ignored, or abused extensively. It is not an easy matter to evaluate the specificity of an inhibitor under various conditions, particularly when the necessary data are lacking, but it is hoped that at least a provisional picture has been presented in some instances.

Certain aspects of metabolism (e.g. glucose utilization, respiration, photosynthesis, protein synthesis, or oxidative phosphorylation) and cellular activity (e.g. active transport, membrane potentials, movement, mitosis, or proliferation) are obviously of general significance, and the effects of inhibitors on these have been emphasized. This is not to say that other pathways or functions are unimportant, and indeed where necessary they have been treated as adequately as possible, but one cannot discuss all the actions of each inhibitor, so that some compromises must be made.

A major use of inhibitors is in the attempt to correlate cellular functions with particular enzymes or metabolic pathways, and for this reason, as well as the fact that this represents one of the most fascinating aspects of inhibitor study, these correlations have been discussed fully if the information has been available, and the effects on certain organisms or processes have often been given in the hope that some correlation will emerge or further work will be stimulated. It is believed that conceiving inhibitor actions in terms of deviations in the energy flow is of some value although an accurate formulation of this must await the development of a new terminology.

It is simpler to restrict the treatment of an inhibitor's action to a particular organism or tissue, but it is felt that a great deal may be learned from comparative inhibitor enzymology. Therefore, in the tables, the effort has been made to present the results from as many sources as possible for a particular enzyme or metabolic pathway since by doing this one is better able to see the great extent of the variability in responses; only a distorted view is obtained if a limited range of action is considered.

Paradoxical actions have been both the despair and delight of scientists in many fields, and it is recognized that some of our finest theories have originated in the observation and study of anomalies. There is an inherent desire in most of us to eliminate anomalies and perhaps devote a good deal
of effort to this, since we feel that an anomaly really is something we would expect if we knew the system or mechanism better, or as Henry Miller has said in the "Tropic of Capricorn," "confusion is a word we have invented for an order which is not understood." I have thus brought up certain so-called anomalies, not only for their interest but again because they often stimulate deeper investigation, although at present they may to some only confuse the picture.

Many of the results have been put into tabular form, first because this is the most efficient way of presenting certain types of data, second because such simple observations are often the sole information on the inhibitors provided in the reports, third because this allows a more convenient comparison of results (e.g., for those interested in possible phylogenetic relationships, for which reason the source organisms have usually been given in the classic taxonomic sequence, or for studying the variability in responses on a comparative basis), fourth because this is the clearest way to provide information from which specificity may be evaluated, and fifth because these tables may serve as reference sources for those interested in the actions of a particular inhibitor on a certain enzyme or organism. There is much more in these tables than anyone can assimilate or understand or interpret today, but it is these data which could possibly contribute to some idea or concept if placed against the proper experience or background. Nothing makes some data look more miserable or incomplete than putting them in tables, but perhaps this is an asset, since it shows what is missing, what should have been done, and what more there is to do. A great deal of information could not be included in the tables, for, although some of them look formidably long, they represent only a fraction of what is available in reports. One tries to include only that which is important, but the definition of this word becomes more difficult as one applies it. There are so many very specialized and unique enzymes being isolated and studied these days, that it becomes more of a problem each year to determine which of the enzymes are generally significant. An enzyme which at first sight might seem esoteric, if for no other reason than its gargantuan name, implying a specificity of catalysis incommensurate with anything but a very limited role in metabolism, may well be of great importance in a particular pathway, a pathway perhaps as yet undiscovered. Every enzyme is of some importance to some organism or tissue, or it would not be there. And we often take a limited viewpoint; one of the numerous enzymes in the pathway of steroid biosynthesis is recognized as important in cholesterol or adrenal corticoid formation, but it may be equally important to some microorganism in producing steroids which function
in their membranes, the inhibition of the formation of which could lead to a suppression of growth. In view of the past history of science, anyone is presumptuous to claim they can distinguish what is important from what is not — we have to do this much of the time, of course, but we should realize we are presumptuous. There are probably some errors in the tables, since it is often difficult to determine exactly the conditions used; one is sometimes referred to a previous report, but cannot be certain that all the conditions have been maintained throughout the work. One must often guess a parameter from other work the investigators have done, and sometimes calculate results from heterogeneous data. There has been a good deal of calculation, and recalculation, and averaging, and I take full responsibility for anything right or wrong I may have done. A number of curves have been replotted or data represented in a way that differs from that of the original investigator, and I fully realize that this usually results in nothing but animosity.
CONVENTIONS

The naming of enzymes is not an easy task. On the one hand, there are the more trivial names with their occasional confusions — on the other, there are the official names in the “Report of the Commission on Enzymes” (1961) which are reasonably precise but often unwieldy. I have usually chosen the former because I feel most readers will recognize these more readily, but frequently I have taken an intermediate course which probably will not please anyone. It is much more accurate to write NADH:menadione oxidoreductase than to use the designation NADH oxidase or NADH dehydrogenase, since the former name indicates the substrate and acceptor used. In addition it is cumbersome to use D-xylulose-5-phosphate D-glyceraldehyde-3-phosphate-lyase (phosphate-acetylating) instead of phosphoketolase, yet there is no doubt that this longer term accurately describes the enzyme. There are also preferences in nomenclature, for various reasons. I never cared much for the term invertase; I prefer to call it β-fructofuranosidase, although it is clumsier, but not as much so as β-D-fructofuranoside fructohydrolase. In other instances the older and shorter names are more pleasing to me and I imagine to others. I have tried to use enzyme names which, at least, can be found in the index of the “Report of the Commission on Enzymes,” and some cross referencing of names has been included in the index. There are certain instances of inconsistency which I do not particularly regret.

As in the first volume, concentrations have been given as millimolar (mM) except when designated otherwise, and in other matters the conventions given there have been retained.

SYMBOLS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
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<td>absorbance</td>
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<td>E₀</td>
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<tr>
<td>E₀'</td>
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<td>Embden-Meyerhof (pathway)</td>
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<tr>
<td>Symbol</td>
<td>Definition</td>
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<td>------------</td>
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<td>(N)-ethylmaleimide</td>
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<td>pI</td>
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<td>(p)-mercuribenzoate ion</td>
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<td>(p)-mercuriphenylsulfonate ion</td>
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<td>p-Q</td>
<td>(p)-benzoquinone</td>
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<td>p-QH_2</td>
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<td>9,10-PAQ</td>
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<td>3-PGDH</td>
<td>3-phosphoglyceraldehyde dehydrogenase</td>
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</tbody>
</table>
Iodoacetate and related compounds have been used extensively for many years to inhibit the metabolism of glucose and other carbohydrates to pyruvate aerobically, or to lactate, ethanol, and various fermentative products anaerobically, and iodoacetate has often been considered to be a rather specific inhibitor of glycolysis. Specific inhibitors of this pathway of metabolism would be valuable for many studies attempting to evaluate the role of glycolysis in cellular growth or function, and thus it is important to establish as clearly as possible how well iodoacetate can achieve such a selective effect and to determine the optimal conditions for its use. Iodoacetate has also been classified as an SH reagent by reason of its ability to alkylate these groups and we must consider carefully if this is the sole, or most important, reaction occurring under the usual experimental conditions. Iodoacetate has fallen into disfavor among certain enzymologists because it frequently does not react as readily with enzyme SH groups as do the mercurials and some other SH reagents. However, it is exactly this behavior which makes iodoacetate a valuable tool in metabolic studies since it allows a degree of selectivity to be exerted. The mercurials are without question more reliable for the detection of enzyme SH groups but have little value in work on the cellular level inasmuch as so many components, both enzymic and nonenzymic, possess SH groups and react readily with the mercurials. The emphasis in this chapter, therefore, will be placed on the metabolic and functional aspects of iodoacetate action.

HISTORICAL DEVELOPMENT

The early development of the alkylating type of SH reagent involved almost exclusively the use of bromoacetate. Steinauer (1874) in Berlin was particularly interested in the actions of bromide and organic bromine compounds (e.g., bromalhydrate and bromoform) and, while investigating these substances, found that bromoacetate injected subcutaneously in frogs in doses of 5–10 mg kills the animals in 2–3 hr, and that the animals suffer a progressive respiratory and cardiac depression. He concluded that the car-
1. IODOACETATE AND IODOACETAMIDE

diotoxic action is mainly responsible for the death of the frogs. He also
observed, however, that the muscular system is affected: weakness and
paralysis occurred so that spontaneous movement was inhibited and the
animals did not right themselves when placed on their backs. Rabbits be-
haved very similarly following subcutaneous doses of 200–500 mg/kg and
it was noted that the heart became stiff in contracture soon after death.*
He further proved that the cardiac depression is exerted on the heart rather
than through the vagi by demonstrating that section of these nerves did
not significantly modify the effect. Dibromoacetate acted less potently and
tribromoacetate scarcely at all. Pohl (1888), in Prague, took up this work
and emphasized the action on skeletal muscle, finding in frogs and rabbits
that a state of rigor followed the earlier weakness, paralysis, and muscle
fibrillation; indeed, his frogs became so stiff they could be held out hori-
zontally like a stick. The first experiment, the results of which are shown
in the tabulation below, indicated the sequence of events following the
injection of 50 mg of bromoacetate into the lymph sac of a large frog.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Spontaneous movements cease</td>
</tr>
<tr>
<td>5</td>
<td>Animal remains on back when so placed</td>
</tr>
<tr>
<td>9</td>
<td>Muscle fibrillating and cardiac rate reduced</td>
</tr>
<tr>
<td>15</td>
<td>Limbs extended stiff in contracture</td>
</tr>
<tr>
<td>27</td>
<td>Cardiac standstill</td>
</tr>
</tbody>
</table>

He then showed that the action is peripheral on the muscles by the follow-
ing experiments. Arterial ligation of one leg prevented the development of
rigor in the muscles of that leg at a time when rigor occurred in the normal
leg. A denervated muscle would not go into contracture but if stimulated,
either through the nerve or directly in curarized preparations, the contrac-
ture soon appeared, indicating that the development of rigor depends on a
certain degree of muscle activity. It was known that muscles in rigor are
often acid, but Pohl admitted that he could detect no acidity with litmus
paper in bromoacetate-treated muscles in rigor, an observation the signifi-
cance of which could not be appreciated until many years later. It is unex-
plained why Pohl found iodoacetate to be relatively ineffective, but possi-
ibly his sample of iodoacetate was defective.

* Steinauer apparently first described the actions of bromoacetate on mammals:
"Die Kaninchen blinzeln mit den Augenlidern, ihre Extremitäten glitten aus, ihre
Schnauze sank auf die Unterlage, während sie auf Kneifen noch reagirten; die Thiere
ruhten mit dem Rumpfe vollständig auf der Unterlage."
The studies of Embden and his collaborators in Germany from 1914 to 1921 showed that the increased acidity of muscles stimulated under anaerobic conditions, or in contracture, is due to the accumulation of lactic and phosphoric acids derived from the phosphorylated products of carbohydrate metabolism, and it was believed furthermore that contractions and contractures might be due to this acid produced, the acidity altering the colloidal state of the muscle proteins. Von Fürth (1919) in his review of muscle physiology stated that bromoacetate contracture is due to an explosive formation of lactic acid. The reports by Schwartz and Oschmann (1924, 1925) that neither lactic nor phosphoric acid accumulated in muscles put into contracture by bromoacetate, indicating that this type of contracture is different from others, went without notice.

The turning point in this aspect of muscle physiology came with the work of Lundsgaard (1930a) in the Institute for Medical Physiology at the University of Copenhagen, and these studies not only established some basic concepts of muscle contraction but introduced iodoacetate into general use. Lundsgaard confirmed the effects of bromoacetate in frogs and rabbits but found iodoacetate to be more potent. The intravenous injection of iodoacetate at 50 mg/kg into rabbits produced a sudden development of general muscle rigor before death, the animals becoming “starr wie ein Stück Holz,” whereas frogs could live for several hours after the onset of rigor. The basic contribution of Lundsgaard lies in his demonstration that iodoacetate inhibits the formation of lactate in muscles under a variety of conditions, and that muscles so poisoned retain the ability to contract until they go into rigor. He found frog muscles in situ to contain 57 mg% lactate normally but only 39 mg% after injection of iodoacetate. Muscles standing for 2 hr contain high concentrations of lactate (649 mg%) whereas iodoacetate-treated muscles under the same conditions contain very little lactate (27 mg%). If the muscles removed from iodoacetate-poisoned frogs were stimulated to contract, no more lactate was found in them than in those muscles allowed to rest. As the muscles were stimulated, the contractile amplitude decreased and contracture soon ensued although lactate was not formed, as shown in the accompanying tabulation. The poisoned muscle will con-

<table>
<thead>
<tr>
<th>Muscle</th>
<th>Lactate concentration (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated</td>
</tr>
<tr>
<td>Resting</td>
<td>28.3</td>
</tr>
<tr>
<td>Contracting</td>
<td>83.9</td>
</tr>
</tbody>
</table>

tract without formation of lactate and do as much work as a normal muscle producing 50–60 mg% lactate. If lactate is not responsible for contractions
and contractures, what is? Lundsgaard found that phosphagen (mainly creatine phosphate) is only partially broken down during activity in normal muscles (61 mg% to 46 mg%) but that in iodoacetate-treated muscles it is broken down completely (57 mg% to 0 mg%). When the phosphagen reaches a low level, contracture ensues. He postulated that the breakdown of phosphagen is perhaps the source of energy for muscle contraction and that lactate is possibly used to resynthesize phosphagen. It must be realized that at this time the sequence of glycolytic reactions had not yet been established and the intermediates between hexose diphosphate and lactate were unknown. It is interesting that Lundsgaard observed in iodoacetate-poisoned muscle that the phosphate from phosphagen split during activity does not appear as inorganic phosphate but as hexose phosphates, which markedly increase in the muscles, indirectly indicating a block in the breakdown of hexose phosphates. The incidental observation of Pohl (1888) that muscles in bromoacetate contracture show no increase in acidity could now be explained and the concepts of the role of lactate in muscle contraction were markedly altered.*

Numerous investigations, stimulated by these observations, were carried out during the next 3 or 4 years, during which time with the help of the inhibitors fluoride and iodoacetate, perhaps the most important single tools, the glycolytic pathway was worked out and the site of action of iodoacetate localized. The major portion of this work was done in the Institute for Physiology at the Kaiser-Wilhelm Institute in Heidelberg under the direction of Meyerhof, Lohmann, Lipmann, and others. This subsequent development will now be outlined very briefly. Lundsgaard (1930 b) reasoned that iodoacetate has no inhibiting action on the formation of hexose phosphates from glycogen or on the breakdown of phosphagen in muscle, and thus must act on the enzymic splitting of glucose ("auf die anoxybiontische enzymatische Spaltung der Monohexosen eine spezifisch aufhebende Wirkung ausübt"). In order to study this he turned to glucose fermentation in yeast and found that 0.54–1.1 mM iodoacetate inhibits the utilization of glucose almost completely. No inhibition of invertase, amylase, or catalase by iodoacetate at much higher concentrations was found, so that Lundsgaard concluded that iodoacetate inhibits "zymase" (the name then given to the enzyme complex responsible for the breakdown of glucose). Inhibition of fermentation in cell-free extracts of yeast was well demonstrated by determinations of the CO₂ production. Another important observation was that iodoacetate requires some time to achieve its maximal inhibition; even 0.4 mM inhibits completely after 2 hr incubation.

* It should be pointed out that Lundsgaard was unaware of the earlier work of Schwartz and Oschmann on muscle contracture when he reported his first studies. These earlier investigations, however, were not concerned with the relationship between lactate and contraction, but only with the development of rigor.
At the same time, Lundsgaard (1930 c) believed that iodoacetate could effect a separation of anaerobic glycolysis and respiration, since 1.1 mM iodoacetate inhibits the former completely and has no effect on yeast respiration, a point which received much attention later, as we shall see. Iodoacetate also acts more potently to suppress the CO₂ production under anaerobic conditions than aerobically. Lundsgaard also first demonstrated the fall in R.Q. (1.38 to 0.99) brought about by iodoacetate as the utilization of glucose is inhibited in yeast. A rather specific effect on glycolysis was indicated in the work of Yamasaki (1930), who showed that iodoacetate strongly inhibits the fermentation of hexose diphosphate and has little effect on pyruvate fermentation. A similar result was obtained by Krebs (1931) in mammalian tissues in which iodoacetate at 0.3 mM inhibits anaerobic glycolysis and respiration in the presence of glucose while inhibiting the O₂ uptake from lactate relatively little (see accompanying tabulation). Two

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% Inhibition of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anaerobic glycolysis</td>
</tr>
<tr>
<td>Testis</td>
<td>93</td>
</tr>
<tr>
<td>Brain</td>
<td>92</td>
</tr>
<tr>
<td>Jensen sarcoma</td>
<td>93</td>
</tr>
</tbody>
</table>

basic problems thus existed: (1) can iodoacetate separate glycolysis from respiration as Lundsgaard had believed, and (2) can lactate counteract the inhibitory effect of iodoacetate on respiration? These problems were quite important at that time and much work was done to elucidate the complexities; knowing what we do today about metabolism, the answers being fairly clear, it is often difficult to appreciate the concern over these matters. Krebs in finding that iodoacetate inhibits the respiration in animal tissues in the presence of glucose opposed the idea that separation of glycolysis and respiration could be achieved, and believed there to be two phases in glucose metabolism: (1) a first anaerobic phase in which glucose is metabolized to lactate, and (2) a second aerobic phase wherein the lactate is utilized. Substitution of pyruvate for lactate in this formulation would accord with modern concepts. Thus if iodoacetate inhibits the first phase, it should also inhibit the respiration or second phase, since it is dependent on the first phase for the supply of oxidizable substrate. This would be true if glucose were the sole source for the respiration, but many cells oxidize various endogenous substrates even in the presence of glucose and this could account for an iodoacetate-resistant fraction of respiration. One can say that the respiration ultimately due to the formation of pyruvate by the
glycolytic pathway is especially sensitive to iodoacetate and may be inhibited without greatly affecting other fractions of the \( O_2 \) uptake, if the proper conditions are established. Meyerhof and Boyland (1931) showed that lactate oxidation is not markedly interfered with in iodoacetate-poisoned muscle, and found that pyruvate stimulates the respiration even more effectively in the presence of iodoacetate. Muscle poisoned with iodoacetate will contract longer when lactate is provided and the lactate disappears (Mawson, 1932); this showed that lactate can be used for the resynthesis of phosphagen. In *Bacterium delbrückii* 0.17 mM iodoacetate inhibits glucose respiration 61% and lactate respiration not at all. All recent work, if properly done, has confirmed these original general conclusions. Pyruvate or lactate oxidation may thus proceed in the presence of iodoacetate but, if their formation depends on glycolysis, the respiration will be depressed.

The determination of the site of iodoacetate action in the glycolytic pathway proceeded concurrently with the elucidation of the sequence. The emphasis on the fact that fluoride and iodoacetate inhibit at different loci (Lohmann, 1931) was very important in the analysis. Meyerhof and Kiesling (1933) showed that whereas fluoride blocks the formation of pyruvate from P-glycerate, iodoacetate is inactive; iodoacetate, on the other hand, blocks the reduction of pyruvate to lactate when coupled with the oxidation of phosphoglyceraldehyde. Furthermore, in the presence of iodoacetate an easily hyrolyzable ester appears during the breakdown of glucose or hexose phosphates and, after isolating phosphoglyceraldehyde from muscle, they showed that its metabolism is blocked by iodoacetate. Embden and Deuticke (1934) found that fructose-diP no longer forms P-glycerate and glycerido-P in poisoned muscle, and believed they could isolate some phosphoglyceraldehyde as a result of the block. The reaction coupling pyruvate reduction with phosphoglyceraldehyde oxidation is inhibited potently by iodoacetate but the site of the block was not known. Green and Brosteaux (1936) found lactate dehydrogenase to be quite resistant to iodoacetate, so that the sensitive reaction seemed to be the phosphoglyceraldehyde dehydrogenase. However, the picture was temporarily confused by the report of Dixon (1937) that phosphoglyceraldehyde dehydrogenase is not very sensitive to iodoacetate; indeed, he found alcohol dehydrogenase (which plays a role comparable to the lactate dehydrogenase in alcoholic fermentation) to be more sensitive and felt this to be the site of inhibition in yeast fermentation. That the principal point of attack is the phosphoglyceraldehyde dehydrogenase in all cases was finally proved by Adler et al. (1938), although the demonstration of inhibition of the purified enzyme had to await the work of Cori et al. (1948). The inhibition of glucose utilization, lactate or ethanol formation, and respiration due to glucose, as well as the accumulation of certain phosphate esters, could now be readily explained on the basis of a fairly specific block of the step in which phosphoglyceraldehyde
is oxidized and in which a high-energy phosphate is incorporated. Much work since that time has confirmed the sensitivity of phosphoglyceraldehyde dehydrogenase to iodoacetate, but has also produced evidence that the inhibition is perhaps not as selective as originally believed unless great care is taken in choosing the experimental conditions, especially the concentration of iodoacetate.

**CHEMICAL PROPERTIES**

Iodoacetic acid is a rather reactive and unstable substance, due primarily to the relative weakness of the C—I bond, so that it slowly decomposes to release I⁻ and I₂, as is evident in most commercial samples. These reactions are accelerated in aqueous media, and by light or increase in temperature. It has long been known that heating aqueous solutions of the halogen acids leads to a hydrolysis:

\[ XCH₂COO⁻ + H₂O ⇄ HOCH₂COO⁻ + H^+ + X^- \]

forming glycolate and the halide anion. Under physiological conditions of pH and temperature these reactions are slow and not important in experiments of several hours’ duration; however, they are partly responsible for the decomposition of stored solutions of the halogen acetates, and may be of some significance in long-term experiments. The activation energy for the hydrolyses is quite high — 23.1 kcal/mole for bromoacetate and 22.6 kcal/mole for iodoacetate — so the reactions are very temperature-dependent (Kunze and Merkader, 1940). At 70° and 100 mM, the half-reaction times are 10 hr for bromoacetate, 25 hr for chloroacetate, and 61 hr for iodoacetate (Drushel and Simpson, 1917). In 1 hr the reactions would proceed 7%, 3%, and 2%, respectively. The kinetics of hydrolysis are complex and various salts exert catalytic effects (Dawson and Dyson, 1933). Hydrolysis is usually somewhat faster for the halogen acetates than for the undissociated acids (Dawson, 1935). Exchange reactions of iodoacetate with other halides may occur:

\[ ICH₂COO⁻ + Cl⁻ ⇄ ClCH₂COO⁻ + I^- \]

and the rates are quite appreciable at physiological temperatures (Wagner, 1925). Such exchange reactions, indicating the instability of the iodine atom, have recently been studied by van der Straaten and Aten (1954) using I¹³¹:

- \[ ICH₂COOH + I^{¹³¹} ⇄ I^{¹³¹}CH₂COOH + I \]  
  \[ \text{(A)} \]
- \[ ICH₂COO⁻ + I^{¹³¹} ⇄ I^{¹³¹}CH₂COO⁻ + I \]  
  \[ \text{(B)} \]
- \[ ICH₂COOCH₃ + I^{¹³¹} ⇄ I^{¹³¹}CH₂COOCH₃ + I \]  
  \[ \text{(C)} \]
- \[ ICH₂COOH + I^{¹³¹} ⇄ I^{¹³¹}CH₂COOH + I₂ \]  
  \[ \text{(D)} \]
1. IODOACETATE AND IODOACETAMIDE

The reaction rate constants for (A) and (C) are similar, and about $8.7 \times 10^{-3}$ liters/mole/sec, with an activation energy around 16–17 kcal/mole, corresponding to an exchange rate of 0.0234 mole/mole/min. Reaction (B) is about one-tenth and reaction (D) about one-seventeenth as rapid. A reductive reaction with iodoacetate:

$$\text{ICH}_2\text{COO}^- + \text{H}^+ + 2\text{e}^- \rightarrow \text{CH}_2\text{COO}^- + \text{I}^-$$

may also occur under certain conditions and is the basis for the polarographic determination (Brdička, 1936). The stability in tissue preparations is of course much less, due to reaction with several components; thus, bromoacetate in ox blood at 37° is 23% reacted in 2 min, 50% in 35 min, and 67% in 64 min (Hansen, 1956 b). The methyl and ethyl esters of bromoacetate and iodoacetate hydrolyze very rapidly in solution (Bergmann and Shimoni, 1953) and this should be taken into account whenever these esters are used to facilitate penetration.

Iodoacetic acid which has been obtained commercially or which has been standing for some time should be tested for free iodine with starch, and repurified if a positive test is given. Some enzymes are very sensitive to iodine (see Chapter II-5).* Lotspeich and Peters (1951) found that isocitrate dehydrogenase is not inhibited by recrystallized iodoacetate but is by discolored samples, which could explain some of the earlier discrepancies. Iodoacetic acid may be readily recrystallized from carbon tetrachloride (Hellström, 1931), or dissolved in benzene and precipitated with petroleum ether. The preparations of the sodium salts of bromoacetate and iodoacetate have been described by Drushel and Simpson (1917) and Goldberg (1943). Iodoacetamide may be prepared from chloroacetamide and sodium iodide by the method of Anson (1940) and iodoacetamide-I$_{131}$ obtained by the method of Friedman and Rutenburg (1950 a).

The substitution of a halogen atom on acetic acid increases the acid strength by reason of the electronegative nature of these atoms (Table I-1). β-Substitution in propionic acid has less effect because of the greater distance between the halogen and the carboxyl group. Thus iodoacetic and bromoacetic acids are quite strong acids so that at physiological pH’s there is very little of the undisassociated species, which may be of importance in work with cells relatively impermeable to anions. The ratio (iodoacetate$^-$)/(iodoacetic acid) at pH 7.4 is 18,600 and, at a total concentration of 1 mM, (iodoacetic acid) = $5.4 \times 10^{-5}$ mM. Of possible significance in permeabi-

* Cross references to chapters, figures, equations, etc., in other volumes of this treatise will appear in the following manner: Chapter I-6; Fig. II-6-7; Eq. II-3-1; etc. The Roman numeral refers to the volume; the first Arabic numeral to the chapter number; and the second Arabic numeral to the figure or equation number, as the case may be.
Table 1-1
IONIZATION CONSTANTS$^a$ FOR HALOGEN-SUBSTITUTED ACIDS

<table>
<thead>
<tr>
<th>Acid</th>
<th>$pK_a$</th>
<th>$K_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic</td>
<td>4.73</td>
<td>$1.86 \times 10^{-5}$</td>
</tr>
<tr>
<td>Fluoroacetic</td>
<td>2.66</td>
<td>$2.19 \times 10^{-3}$</td>
</tr>
<tr>
<td>Chloroacetic</td>
<td>2.81</td>
<td>$1.55 \times 10^{-3}$</td>
</tr>
<tr>
<td>Bromoacetic</td>
<td>2.85</td>
<td>$1.41 \times 10^{-3}$</td>
</tr>
<tr>
<td>Iodoacetic</td>
<td>3.12</td>
<td>$7.59 \times 10^{-4}$</td>
</tr>
<tr>
<td>Dichloroacetic</td>
<td>1.26</td>
<td>$5.50 \times 10^{-2}$</td>
</tr>
<tr>
<td>Trichloroacetic</td>
<td>0.7</td>
<td>$2.0 \times 10^{-1}$</td>
</tr>
<tr>
<td>Propionic</td>
<td>4.85</td>
<td>$1.41 \times 10^{-5}$</td>
</tr>
<tr>
<td>$\beta$-Chloropropionic</td>
<td>3.97</td>
<td>$1.07 \times 10^{-4}$</td>
</tr>
<tr>
<td>$\beta$-Bromopropionic</td>
<td>3.99</td>
<td>$1.02 \times 10^{-4}$</td>
</tr>
<tr>
<td>$\beta$-Iodopropionic</td>
<td>4.03</td>
<td>$9.33 \times 10^{-5}$</td>
</tr>
</tbody>
</table>

$^a$ These values have been corrected as far as possible for $37^o$ and an ionic strength of 0.15.

Lability considerations is the fact that both iodoacetic and bromoacetic acids tend to concentrate somewhat in an oil phase; thus the partition coefficients are 2.90 for iodoacetic acid and 15.6 for bromoacetic acid using olive oil (Bodansky and Meigs, 1932), and 2.4 for bromoacetic acid using octanol (Collander, 1951).

Reactions with Thiols

By 1910 it was recognized that iodoform is broken down in the tissues and by cell-free preparations. Thunberg (1911 b) demonstrated that iodine is set free from iodoform in the presence of thiols and stated, "... Bin ich zu der Auffassung geleitet worden, dass die Giftwirkung einiger Stoffe — der bromsauren und jodsauren Salze — möglicherweise dadurch zu erklären ist, dass sie mit der Sulphydrylgruppe reagieren."* It was also known by organic chemists that halogen acids react with SH groups, as in the Klasen reaction for the synthesis of thiodiglycolate. However, it was not until the importance of iodoacetate in muscle and yeast metabolic studies was recognized that interest in such reactions was stimulated.

* "I have been led to the concept that the toxic actions of certain substances – the salts of bromo and iodo acids – can be explained by their reaction with sulfhydryl groups."
The kinetics of the reactions between halogen acids and thioglycolate

\[-\text{OOCCH}_2\text{SH} + \text{XCH}_2\text{COO}^- \rightarrow \text{-OOCCH}_2\text{SCH}_2\text{COO}^- + \text{Cl}^- + \text{H}^+\]

were thoroughly studied by Hellström (1931, 1933, 1934) in Stockholm. The relative reaction rates are given in the accompanying tabulation. The rate increases with ionic strength — \( k \) is proportional to \( s^{0.27} \) — and with the pH, indicating that the reaction may involve an \( R-S^- \) ion. Some oxidation by iodoacetate to the disulfide, \( \text{-OOCCH}_2\text{SSCH}_2\text{COO}^- \), also occurs, whereas with iodoacetamide this reaction is very slow. Iodoacetate is also able to react with SH groups complexed with metals; the rates of reaction with the mercuric and zinc complexes of thioglycolate are actually greater than with the free SH groups.

The importance of SH groups in enzyme inhibition by iodoacetate was brought to the fore by studies on glyoxalase under the mistaken impression that this is an enzyme involved in the glycolytic pathway, methylglyoxal being considered as the precursor of lactate. Dudley (1931) showed that iodoacetate strongly inhibits glyoxalase and that this occurs \textit{in vivo} following injection of iodoacetate. Lohmann (1931) simultaneously demonstrated that higher concentrations of iodoacetate are required to inhibit glyoxalase than to block glycolysis, and felt that this was not the site of glycolytic inhibition, but showed in the following year (Lohmann, 1932) that glutathione is a cofactor for this enzyme. This led Dickens (1933 a,b) to study the mechanism of inhibition of glyoxalase and he immediately found that the inhibition can be reversed by glutathione; the purified enzyme, with little glutathione to protect it, is inhibited by 0.03-0.1 mM iodoacetate, whereas about 5 times this concentration range is necessary in liver slices. The results indicated a direct reaction of iodoacetate with glutathione, and Dickens found that when neutral solutions are mixed a reaction occurs eliminating iodide:

\[\text{GSH} + \text{ICH}_2\text{COO}^- \rightarrow \text{GSCH}_2\text{COO}^- + \text{I}^- + \text{H}^+\]

The relative rates of reactions are given as: iodoacetate 15.5., bromoacetate 9, and chloroacetate 0.15. Cysteine was also found to react readily with
iodoacetate. Quastel (1933 a) pointed out the importance of glutathione for glycolysis and believed it might represent the site of attack for iodoacetate.

The SH groups on proteins and enzymes vary widely in reactivity with SH reagents (Chapter II-5), and part of this is not due to steric factors introduced by the complex protein structure since simple thiols also vary in how rapidly they react with iodoacetate. Michaelis and Schubert (1934) reported that under specified conditions the reaction of iodoacetate with cysteine is about 70% complete in 10 min, with thioglycolic anilide in 30 min, and with thioglycolate in 3 hr. Smythe (1936) investigated this matter further and determined the half-reaction times at pH 7.1, from which the relative rates in the accompanying tabulation were calculated. The half-

<table>
<thead>
<tr>
<th>Thiol</th>
<th>Relative reaction rate with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iodoacetate</td>
</tr>
<tr>
<td>Thioglucose</td>
<td>1.38</td>
</tr>
<tr>
<td>Thiosalicylate</td>
<td>1.33</td>
</tr>
<tr>
<td>Cysteine</td>
<td>1.00</td>
</tr>
<tr>
<td>Glutathione</td>
<td>0.56</td>
</tr>
<tr>
<td>Thioglycol</td>
<td>0.16</td>
</tr>
<tr>
<td>Mercaptoethane</td>
<td>0</td>
</tr>
</tbody>
</table>

reaction time for iodoacetate and cysteine is 1.12 min. It appears that, under these conditions, iodoacetamide always reacts somewhat more rapidly than iodoacetate, but Hellström found iodoacetate to react faster with thioglycolate (see page 10). It may also be noted that no reaction at all occurs with GSSG. Comparison of the rates of reaction of the various halogen acids with cysteine was made by Barron (1951) at pH 7.4 and 28°, indicating that while iodoacetate and bromoacetate are not very different,

<table>
<thead>
<tr>
<th>Relative rate of reaction with cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodoacetate</td>
</tr>
<tr>
<td>Bromoacetate</td>
</tr>
<tr>
<td>Chloroacetate</td>
</tr>
<tr>
<td>Fluoroacetate</td>
</tr>
</tbody>
</table>

as has been found generally, chloroacetate and fluoroacetate are very unreactive. This has been confirmed more recently by Mayer (1957), who found iodoacetate to react 155 times more rapidly with cysteine than does
chloroacetate at pH 6.8 and 25°; indeed, it requires 44 hr to block 50% of the SH groups with chloroacetate. The iodoacetamides react quite slowly with glutathione, indicating the importance of the inductive effects in promoting reactivity, and suggesting that the effects observed with the iodoacetamides are not due to reaction with SH groups (Schroeder et al., 1933 b). It has generally been considered that reaction of iodoacetate with proteins is entirely through the cysteine residues with respect to SH groups. However, Gundlach et al. (1959 b) have shown that the carboxymethylsulfonium salt of methionine is formed at a rapid rate throughout the pH range 2–8.5, and thus that methionine residues must also be visualized as points of attack.

The relationship between the pH and the reactivity of SH groups with iodoacetate is a very important matter in determining which groups on enzymes are alkylated (see page 33), and we shall now examine the evidence obtained with simple thiols. Rapkine (1933 a, 1936) reported that the reaction of iodoacetate with cysteine and glutathione accelerates as the pH is raised, especially around pH 7, indicating that the ionization of the SH groups is the critical factor and that the $S^-$ ion is the reactive form. Schroeder et al. (1933 b) found almost no reaction between iodoacetate and glutathione at pH 4.5 but that the rate increases as the pH is raised to 8.5. Similarly with bromoacetate, the reaction with cysteine is very slow at pH 5.5, faster at pH 6.5, and fastest at pH 7.2 (Hansen, 1956 b), while the kinetics of the reaction between chloroacetamide and mercaptoacetate indicate the $S^-$ form to be the reactive species (Lindley, 1960). A very interesting relationship was found by Smythe (1936) when he compared the relative rates of reaction of thiols with iodoacetate and iodoacetamide at different pH’s. As the pH was dropped from 7.1 to 6.1, the rates with both were lower, but the decrease was greater with iodoacetate; thus the ratio of the rates with iodoacetamide and iodoacetate was 1.92 at pH 7.1, and 4.02 at pH 6.1 (means for all the thiols tested). It would be interesting to know if at higher pH the difference between iodoacetate and iodoacetamide disappears, which might mean that both react with the $S^-$ form equally, but that iodoacetamide reacts relatively more rapidly with the undissociated SH group. Benesch and Benesch (1957) reported that iodoacetamide reacts

<table>
<thead>
<tr>
<th>pH</th>
<th>Relative rate of reaction with cysteine</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.6</td>
<td>0.14</td>
</tr>
<tr>
<td>6.3</td>
<td>0.28</td>
</tr>
<tr>
<td>7.0</td>
<td>1.00</td>
</tr>
<tr>
<td>7.53</td>
<td>1.40</td>
</tr>
<tr>
<td>8.36</td>
<td>2.10</td>
</tr>
</tbody>
</table>
quite readily with glutathione at pH 9 where most of the SH groups are
dissociated, this being used as a basis for SH group determination by re-
cording of the H\(^+\) produced during the reaction.

We may briefly turn to the question whether glutathione can be alkylated
by iodoacetate intracellularly. Schroeder et al. (1933 b) demonstrated that
appreciable amounts of glutathione in yeast can be reacted in the pH range
below 6. The reverse relationship to pH is observed in cellular work in that
inhibition and glutathione disappearance increase as the pH is decreased,
due to the greater penetration of the iodoacetate at the lower pH's (Fig. I-
14-21). Tsen and Collier (1960) have also shown that iodoacetate markedly
depletes erythrocytes of their glutathione, all being reacted after 90 min
exposure to 0.75 mM. Whatever the importance of glutathione and other
free thiols in cellular metabolism and function, it is evident that iodoacetate
can easily deplete them.

Reactions with Amino Groups

The emphasis on iodoacetate as an SH reagent has often obscured the fact
that reaction with amino groups can occur under certain conditions. Neu-
berg and Kobel (1931), in investigating the reason for the disappearance
of iodoacetate in yeast extracts, found that reaction with arginine, alanine,
and aniline occur, releasing iodide. The carboxymethylation of primary
amino groups occurs in two steps:

\[
R{-}\text{NH}_2 + \text{ICH}_2\text{COO}^- \rightarrow R{-}\text{NH}-\text{CH}_2\text{COO}^- + I^- + H^+
\]

\[
R{-}\text{NH}-\text{CH}_2\text{COO}^- + \text{ICH}_2\text{COO}^- \rightarrow R{-}\text{N} + \text{CH}_2\text{COO}^- + I^- + H^+
\]

Both aliphatic and aromatic amines as well as ammonia are reacted. The
carboxymethylated products from glycine and alanine were isolated by Mi-
chaelis and Schubert (1934), but they pointed out that such reactions are
generally slower than with SH groups, so that it is possible to react the SH
group of cysteine before reaction with the amino group has occurred. At
elevated temperatures (80\(^\circ\)) and in alkaline solutions the amino groups may
react quite rapidly, but at physiological pH and temperature it may require
a long time for significant alkylation (the half-reaction time for iodoacetate
and glycine is around 8 hr).

The variation in reaction rate with pH is represented by a sigmoid curve
similar to the titration curve for glycine, indicating that it is the glycinate
anion \(H_2\text{N}--\text{CH}_2--\text{COO}^-\) that reacts; the \(pK_a\) of the amino group is 9.6,
so that below a pH of 8 the rate is very slow (Brdička, 1936). This was
related to the unbonded electron pair possessed by the NH₂ group, the reaction possibly proceeding through an intermediate:

\[ \text{OOC—CH₂—NH₂} + \text{I—CH₂—COO}^- \rightarrow \text{OOC—CH₂—NH—CH₂—COO}^- \]

Even at pH 13, glycine at 33 mM, and iodoacetate at 10 mM, it requires 3 hr for half-reaction. Schubert (1936) pointed out, however, that various amino groups react at quite different rates, and particularly that tertiary amines react faster than primary or secondary amines. Reactions with pyridine, nicotinate, and nicotinamide are fairly rapid; this may in part be related to the lower pKₐ's of these substances (e.g., pKₐ is 5.21 for pyridine) so that a greater fraction is present in the dissociated form. He also pointed out that iodoacetate generally reacts more rapidly than iodoacetamide with amino groups (Table 1-2), in contrast to the situation with SH groups. Again it must be emphasized that even the fastest reacting amines give rates only around 1/400 that for glutathione or cysteine. Histidine reacts slowly with iodoacetate and bromoacetate, presumably in the following way:

\[ \text{CH₂COO}^- \quad \text{HN—CH} \quad \text{HC—N} \quad + \text{XCH₂COO}^- \rightarrow \text{CH₂—C} \quad \text{N—CH} \quad \text{HC—N} \quad + \text{X}^- + \text{H}^+ \]

Despite the slowness of the reaction with free histidine, one histidine residue in ribonuclease reacts quite rapidly (Barnard and Stein, 1959). The studies with amino acids and other simple amines indicate that reactions of iodoacetate with amino groups must be unimportant relative to reactions with SH groups, but we shall see that within the structure of certain proteins the amino groups may be in a special state of reactivity, so that in work with enzymes the amino groups cannot always be ignored. In addition, one must bear in mind the possibility of alkylation of coenzymes, especially the nicotinamide nucleotides.

Reactions with Proteins

Protein SH groups are frequently resistant to iodoacetate and their reactivity is usually increased by denaturation. Denatured and reduced ovalbumin reacts with iodoacetate at pH 7.5 when the iodoacetate concentration is above 2.5 mM, and Rapkine (1933 b) showed that a 1 to 1 reaction between iodoacetate and the SH groups occurs, so that this method can be used to titrate the protein SH groups. The necessity of denaturation for
### Table 1-2

**Rates of Reaction of Iodoacetate and Iodoacetamide with Various Nitrogenous Substances**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Rate of reaction (mmoles of I(^-) formed/min (\times 10^3))</th>
<th>Iodoacetate</th>
<th>Iodoacetamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonia</td>
<td>1.8</td>
<td>2.9</td>
<td></td>
</tr>
<tr>
<td>Methylamine</td>
<td>9.7</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>Dimethylamine</td>
<td>30</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Trimethylamine</td>
<td>130</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>7.5</td>
<td>9.3</td>
<td></td>
</tr>
<tr>
<td>N-Methylglycine</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>N-Dimethylglycine</td>
<td>24</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Creatine</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proline</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>16</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histidine</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>125</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Nicotinate</td>
<td>234</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>Nicotine</td>
<td>300</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>Pyridine</td>
<td>490</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>Glutathione</td>
<td>195,000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Reactions run at pH 6.9 and 30\(^o\). The value for glutathione taken from Dickens (1933 b).

---

rapid and complete reaction has been reported by Rosner (1940), Anson (1940), and Bacq and Fischer (1946), all using ovalbumin. Various methods for titrating protein SH groups or determining the reaction with iodoacetate have been devised. Mirsky and Anson (1935) proposed an "indirect" method, whereby the protein is incubated with 35 mM iodoacetate at pH 7.3 and room temperature for 3 hr. Complete reaction with the SH groups apparently occurs since no cysteine could be found in the hydrolyzates of the proteins treated. Rosner (1940) modified this method by not allowing the protein to coagulate during denaturation and observed an initial rapid reaction with iodoacetate (10 mM at pH 7.3) lasting 5–10 min and due to reaction with SH groups (the nitroprusside reaction was negative after 5 min), followed by a slower reaction perhaps involving the amino groups. Native
ovalbumin does not react discernibly in 5 hr, indicating that both SH and amino groups must be protected, but Anson (1940) reported that iodoacetamide at 19 mM and pH 9 would carboxymethylate 40% of the SH groups in 30 min, even though these groups do not react with porphyrindin or ferricyanide. Anson considered the SH groups in the native protein to be accessible but of low reactivity. A spectrophotometric method was used by Finkle and Smith (1958), based on differences in the absorbance of iodoacetate and iodide at 235 m\(\mu\) and 275 m\(\mu\), and Watts et al. (1961) developed an iodide electrode for measuring the iodide formed in the reactions of iodoacetate with proteins. The latter found, incidentally, that urea-denatured ovalbumin reacts rapidly with 2 equivalents of iodoacetate, and then slowly until a further 9.8 equivalents are used.

It has generally been found that protein amino groups react very slowly or not at all with iodoacetate under the conditions of most enzyme work. Hirade and Ninomiya (1950) attempted to correlate the toxicity of iodoacetate and other alkylating agents with the protein groups reacted, and concluded that SH groups alone were of importance, inasmuch as amino groups reacted very slightly under conditions (pH 8.4 and 30°) where glutathione reacted rapidly and completely. Likewise Habeeb (1960) found \(\beta\)-lactoglobulin to react with iodoacetate below pH 8.5 in a rapid and a slow phase, the latter lasting up to 30 hr and possibly due to amino groups. Of course, under more rigorous conditions it is possible to alkylate amino groups, as shown by Korman and Clarke (1956 a, b), who introduced the carboxymethyl group into amino acids and proteins by using bromoacetate at 200 mM at pH 9 and 35° and incubating for 2–3 days. Such carboxymethylation involves histidine and tyrosine residues and is akin to denaturation since disulfide groups are made available to sulfite. A similar technique was used by Nakatani (1960 a, b) to carboxymethylate hemoglobin. The optimal pH for reaction of histidine groups is pH 9 and tyrosine reacts very slowly under these conditions. Reaction stops within 10 hr at 30° and about 60% of the histidine residues are modified; treatment with urea leads to some further reaction. About one third of the 95 histidine residues in catalase react with bromoacetate by this procedure.

Milder alkylating conditions (incubation with 36 mM iodoacetamide for 1 hr at pH 7.15 and 25°) were used by Guidotti and Konigsberg (1964) to study the role of the SH groups in human hemoglobin. It appears that the amino groups do not react readily since only S-carboxymethylcysteine was found in hydrolyzates. The SH groups which influence heme-heme interaction are located next to the histidine residues linked to the iron atoms of the hemes, but 4 of the 6 SH groups in the native carbonmonoxyhemoglobin do not react with iodoacetamide. It has been thought that these unreactive groups are inside the tetrameric structure, but it was shown that the monomers likewise are not attacked, so it was suggested that the SH groups
are protected as a result of interactions between the \( \alpha \)- and \( \beta \)-chains. Since carboxymethylation of the free SH groups alters heme-heme interactions and the binding of O\(_2\), it is not surprising that oxygenation of hemoglobin affects the reactivity with SH reagents (R. E. Benesch and R. Benesch, 1962). Iodoacetamide does not attack hemoglobin unless it is oxygenated (or complexed with other molecules) under the conditions used, and this was interpreted as due to configurational changes brought about by the oxygenation. Whatever the mechanism, it is interesting that oxygenation does not promote reaction with iodoacetate or \( p \)-mercuribenzoate.

One of the important aspects of the reaction of iodoacetate and related compounds with enzymes is that certain groups are attached to the enzyme, these groups altering the configuration and properties of the enzyme surface. The introduction of negatively charged carboxymethyl groups with iodoacetate modifies the electric field and this must often have some effect on reactions occurring at the active center. Thus it is of interest to inquire how the properties of proteins are changed by alkylation. The studies on keratin by Goddard and Michaelis (1935) are pertinent in this connection, since they determined changes in the isoelectric point following reaction with a variety of agents introducing different groups (see accompanying tabulation). It may also be noted that no detectable reaction with amino

<table>
<thead>
<tr>
<th>Agent</th>
<th>Group introduced</th>
<th>Isoelectric point</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>4.6-4.9</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>Carboxymethyl</td>
<td>3.3-3.6</td>
</tr>
<tr>
<td>Iodoacetamide</td>
<td>Carbamylmethyl</td>
<td>5.0-5.3</td>
</tr>
<tr>
<td>( \alpha )-Bromopropionate</td>
<td>( \alpha )-Carboxyethyl</td>
<td>3.1-3.7</td>
</tr>
<tr>
<td>Iodoethanol</td>
<td>Hydroxyethyl</td>
<td>4.5-4.9</td>
</tr>
</tbody>
</table>

groups occurs even under these rather vigorous conditions (40 hr at pH 9-9.5). Proteins with fewer reactive SH groups than keratin would not be altered so much, but changes in these directions must be expected in all cases. Pillemer et al. (1939) confirmed these results and found no amino groups reacted after 12 hr at pH 8. Not only the isoelectric point but also the solubility and immunological specificity of proteins are changed by reaction with iodoacetate. The coagulation of serum proteins upon heating is abolished by iodoacetate (15 min at 12 m\( M \) and pH 7.4), whereas iodoacetamide enhances clotting (Huggins and Jensen, 1949). After incubation with iodoacetamide, iodoacetate does not produce its usual effect, indicating that both react with the same groups. The difference presumably lies in the negative charges introduced by iodoacetate. Iodoacetamide also alters the nature of the clots, making them more firm and elastic, and increasing the binding of water (Jensen et al., 1950). The inhibiting effect of iodoacetate
was used as a basis for a cancer test, the iodoacetate index being defined in terms of the iodoacetate required and the quantity of protein in the serum (Huggins et al., 1949), but this test is not specific (it is positive in acute inflammation, for example) and has recently been found to be unreliable.

INHIBITION OF ENZYMES

Iodoacetate and related substances have been shown to inhibit many enzymes but in very few instances has an analysis of the inhibition been made. It has generally been assumed that inhibition implies the reaction of iodoacetate with SH groups, but seldom has a quantitative determination of the SH groups actually reacted been made. On the other hand, many enzymes which are usually classified as SH enzymes because of inhibition by other SH reagents, do not react readily with iodoacetate and are not inhibited. We shall consider in this section the inhibition of a few important enzymes and some investigations where an approach to the mechanism has been made. Tables summarizing enzyme inhibitions will be presented in the sections on the different phases of metabolism to facilitate comparison of sensitivities. By no means all of the studies on enzyme inhibition with iodoacetate will be included, but an attempt will be made to select data on enzymes important in the major pathways of metabolism, and to present only data which seem to be accurately obtained. It cannot be overemphasized that the values given in these tables are generally not comparable with one another due to the different conditions under which the experiments were run. Such factors as pH, temperature, period of incubation with the inhibitor, purity of the enzyme, and the presence of cofactors and coenzymes vary greatly and, indeed, are quite often not even stated. The inhibitions are often not quantitative in that they may represent some intermediate inhibition on the way to a maximal inhibition — time curves of inhibition are seldom run — and it is likely that secondary inactivation is commonly superimposed on the effects of primary alkylation. They are to serve more as references for those interested in the behavior of particular enzymes, and at most are indicative of the presence or absence of marked inhibition. Furthermore, the concentrations used are often higher than should be used for blocking glycolysis, and this precludes the possibility of comparing enzyme susceptibilities at more practical concentrations.

3-Phosphoglyceraldehyde Dehydrogenase (3-PGDH)*

The early investigations of Embden and Meyerhof between 1933 and 1935 had shown that the site of the inhibition of glycolysis by iodoacetate is

* The abbreviation 3-PGDH for 3-phosphoglyceraldehyde dehydrogenase will be used throughout this chapter for convenience.
probably at the level of triose-P oxidation. The confusion introduced by Dixon (1937) in finding triose-P oxidation to be less sensitive to iodoacetate than alcohol dehydrogenase — indicating that the site of action in the inhibition of alcoholic fermentation is the alcohol dehydrogenase — was resolved partially by Green et al. (1937 b), who found that in the coupled oxidation-reduction between triose-P and pyruvate the oxidation reaction is the most sensitive, and completely by Adler et al. (1938), whose results differed markedly from those of Dixon in that the oxidation of triose-P was inhibited much more readily than alcohol dehydrogenase by iodoacetate. It was now clear that in the various coupled reactions it is the 3-PGDH which is involved in the inhibition by iodoacetate, and that the block of the formation of lactate or ethanol anaerobically is due to the suppression of the production of NADH required for the reduction of pyruvate or acetaldehyde. Although the inhibition of the oxidation of fructose-1,6-diP (in the presence of aldolase which is insensitive to iodoacetate) by iodoacetate had been demonstrated in several preparations, a direct inhibition of 3-PGDH was first reported by Cori et al. (1948) in a crystalline preparation of the enzyme from rabbit muscle. It is interesting that Warburg and Christian (1939) had crystallized yeast 3-PGDH but had not examined its susceptibility to iodoacetate.

Inhibitions of 3-PGDH by iodoacetate are shown in Table 1-3. One notes first that even within a single source there is great variability in the results, due not only to the different incubation times but also to different temperatures (which run from 0° to 38°), pH's, and degrees of purity. One also suspects, despite the difficulty in comparing the results of different workers, that the enzymes from different sources vary in susceptibility to iodoacetate. In general, the 3-PGDH from microorganisms seems to be less readily inhibited than from animal tissues, although the enzymes from most tissues have never been tested directly. It also seems to be a general rule that NAD-dependent 3-PGDH is more sensitive than NADP-dependent 3-PGDH, as seen in the table from the results of Arnon on the enzymes from spinach leaves. This has also been noted by Gibbs (1952) in preparations of 3-PGDH from peas. We shall see that glycolysis is frequently less inhibited by iodoacetate in plants than in animal tissues, and it may be that the presence of NADP-dependent 3-PGDH is at least partly responsible. There is much indirect evidence that the 3-PGDH from various organisms and tissues is readily inhibited by iodoacetate — Aspergillus niger, Streptomyces coelicolor, Chlorella, sugar beet leaves, heart, brain, retina, etc., but only the enzyme from rabbit muscle has been adequately studied in the pure state and quantitative results obtained. Thus most of our detailed knowledge of the inhibition is based on muscle 3-PGDH and how much of this can be applied to the enzymes from other tissues is not known. It is likely that incubation of 3-PGDH from most animal tissues with iodoacetate at 0.1–0.5 mM for
Table 1-3

INHIBITION OF PHOSPHOGLYCERALDEHYDE DEHYDROGENASE BY IODOACETATE

<table>
<thead>
<tr>
<th>Source of enzyme</th>
<th>Iodoacetate (mM)</th>
<th>Preincubation (min)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>0.1</td>
<td>10</td>
<td>0</td>
<td>Still (1940)</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>10</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Alcaligenes faecalis</td>
<td>0.09</td>
<td>2.5</td>
<td>50</td>
<td>Brenneman and Volk (1959)</td>
</tr>
<tr>
<td></td>
<td>1.8</td>
<td>2.5</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Yeast (methylene blue reduction)</td>
<td>0.33</td>
<td>10</td>
<td>5</td>
<td>Dixon (1937)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>20</td>
<td>46</td>
<td>Adler et al. (1938)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>20</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>20</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Yeast (NAD reduction)</td>
<td>0.33</td>
<td>20</td>
<td>65</td>
<td>Adler et al. (1938)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>20</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Castor beans</td>
<td>1</td>
<td>—</td>
<td>100</td>
<td>Benedict and Beevers (1961)</td>
</tr>
<tr>
<td>Spinach leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAD enzyme</td>
<td>0.081</td>
<td>12</td>
<td>100</td>
<td>Arnon (1952)</td>
</tr>
<tr>
<td>NADP enzyme</td>
<td>0.081</td>
<td>50</td>
<td>90</td>
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</tr>
<tr>
<td>Triatoma infestans (hemipteran)</td>
<td>0.066</td>
<td>—</td>
<td>55</td>
<td>Agosin et al. (1961)</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>—</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>—</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Yoshida sarcoma</td>
<td>0.005</td>
<td>40-70</td>
<td>3</td>
<td>Holzer et al. (1958)</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>40-70</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>40-70</td>
<td>&gt;95</td>
<td></td>
</tr>
<tr>
<td>Rabbit muscle</td>
<td>0.1</td>
<td>1</td>
<td>10</td>
<td>Cori et al. (1948)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>5</td>
<td>100</td>
<td>Cori et al. (1950)</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>—</td>
<td>60</td>
<td>Mackworth (1948)</td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>—</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>2</td>
<td>72</td>
<td>Holzer and Holzer (1952)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>—</td>
<td>50</td>
<td>Holzer (1956)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>—</td>
<td>84</td>
<td>Green et al. (1937 b)</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>15</td>
<td>100</td>
<td>Segal and Boyer (1953)</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>30</td>
<td>100</td>
<td>Racker and Krinsky (1952)</td>
</tr>
<tr>
<td></td>
<td>0.091</td>
<td>—</td>
<td>50</td>
<td>Weitzel and Schae (1959)</td>
</tr>
</tbody>
</table>

*a Iodoacetamide.
15–30 min at room temperature or above will inactivate completely. The inhibition is due to a reaction of the iodoacetate with the apodehydrogenase rather than with NAD, since preincubation of enzyme with the inhibitor leads to initial inhibition when substrate and NAD are added, whereas preincubation of NAD with the inhibitor is ineffective (Adler et al., 1938).

The sequence of reactions catalyzed by 3-PGDH is written by Racker (1961) as:

**Step 1:** $E$–SH + NAD$^+$ $\rightleftharpoons$ $E$–NAD$^-$ + H$^+$

**Step 2:** $E$–NAD$^-$ + R–CHO $\rightleftharpoons$ $E$–S–C–R + NADH

**Step 3:** $E$–S–C–R + HPO$_4^{2-}$ $\rightleftharpoons$ $E$–SH + R–C–O–PO$_3^{2-}$

where R–CHO represents 3-phosphoglyceraldehyde and R–CO–O–PO$_3^{2-}$ represents 1,3-diphosphoglycerate. This is based on the evidence that NAD is bound to the enzyme in part through an SH group and that an acyl enzyme intermediate is formed. Inasmuch as NAD is bound quite tightly to the enzyme, at least in the case of muscle 3-PGDH (dissociation constant around $7 \times 10^{-6}$ M) — the 3-PGDH crystallized from rabbit muscle still retains 2 moles of NAD per mole of enzyme — it is possible that the following scheme, which is analogous to that of Segal and Boyer (1953), may represent the situation more closely in vivo:

**Step 1:** $E$–NAD$^-$ + R–CHO $\rightleftharpoons$ $E$–S–C–R

**Step 2:** $E$–S–C–R + NAD$^+$ $\rightleftharpoons$ $E$–NAD$^-$ + NADH

**Step 3:** $E$–S–C–R + HPO$_4^{2-}$ $\rightleftharpoons$ $E$–S–C–R + R–C–O–PO$_3^{2-}$ + H$^+$
The over-all reactions are the same in both schemes. There are two general types of reaction involved, an oxidoreduction and a transacylation (from the enzyme to phosphate). Since there is evidence that these can occur at different loci on the enzyme, 3-PGDH has been called a double-headed enzyme by Racker (1961), and we shall see that each reaction may be studied alone. For this reason, it is likely that the schemes above do not accurately represent the catalysis. It is actually not known if the same SH group is involved in the binding of NAD, substrate, and iodoacetate and, indeed, we shall discuss evidence that more than one SH group is functional (protection experiments and failure of iodoacetate to inhibit certain reactions catalyzed by 3-PGDH).

Whatever the detailed mechanism of the reactions, it appears certain that iodoacetate carboxymethylates one or more SH groups at or near the active center and thereby interferes with the binding of NAD and substrate. 3-PGDH is faintly yellow in concentrated solution and the color disappears on reaction with iodoacetate; the change in absorption is best determined at 360 m\(\mu\), the approximate maximum of a broad absorption band characteristic of the complex of the apodehydrogenase with NAD (Racker and Krimsky, 1952). Incubation of the apodehydrogenase with iodoacetate also prevents the appearance of the band upon addition of NAD. This has generally been taken to mean that NAD is bound through an SH group reactive with iodoacetate, but does not necessarily follow since the steric interference of a carboxymethyl group might be exerted on the binding of NAD as long as the SH group is in the vicinity of the site. However, thiols such as glutathione complex with NAD nonenzymically, resulting in light absorption in a band centering around 335 m\(\mu\). The reduction in the absorption at 360 m\(\mu\) brought about by iodoacetate is certainly parallel to the loss of catalytic activity (see accompanying tabulation) (Racker, 1954 b;

<table>
<thead>
<tr>
<th>Iodoacetate (equivalents)</th>
<th>Optical density at 360 m(\mu) (%)</th>
<th>Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>66</td>
<td>69</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>28</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Racker and Krimsky, 1958). This reconstituted muscle 3-PGDH contained 3 moles of NAD per mole of enzyme. It is interesting that although iodoacetate reacts rapidly with the enzyme-NAD complex, iodoacetamide reacts very sluggishly, indicating some importance of the COO\textsuperscript{-} group and providing some basis for the occasional observations that iodoacetamide is not as effective an inhibitor of glycolysis as iodoacetate.
The SH groups of the active center may belong to glutathione or some related substance, bound in peptide linkage, and glutathione has been isolated from purified 3-PGDH (Krimsky and Racker, 1952; Segal and Boyer, 1953; Racker, 1954 b). However, this has not been established and several facts, such as the much greater reactivity of the enzyme SH groups to iodoacetate, are not readily explained. Incubation of 3-PGDH with iodoacetate reduces the yield of glutathione obtained on tryptic digestion, as expected if glutathione were involved in the inhibition, but no labeled glutathione could be found following reaction with iodoacetate-C^14. Nevertheless, labeled acetylglutathione was isolated from 3-PHGD allowed to react with labeled acetyl-P (Krimsky and Racker, 1954). Recent work has proved that the three reactive SH groups belong to cysteine residues in the polypeptide structure of the apodehydrogenase. Treatment of rabbit muscle 3-PGDH with iodoacetate-1-C^14 and subsequent digestion provided a peptide of the following structure:

\[
\begin{align*}
\text{NH}_2 & \quad \text{NH}_2 \\
\text{Ileu-Val-Ser-Asp-Ala-Ser-Cys-Thr-Thr-Asp-Cys-Leu-Ala-Pro-Leu-Ala-Lys} \\
\text{CH}_2 & \\
\text{COO}^- 
\end{align*}
\]

and all the labeling occurred in this peptide (Harris et al., 1963). It is likely that 3-PGDH contains three identical polypeptide chains, each associated with an active site. The other cysteine SH group in the peptide above does not react with iodoacetate unless the enzyme is denatured. The esterolytic activity of 3-PGDH, with p-nitrophenylacetate as the substrate, is readily inhibited by iodoacetate (Taylor et al., 1963), so it was assumed that this catalysis proceeds at or near the site at which 3-phosphoglyceraldehyde is reacted, but that p-nitrophenylacetate acetylates the enzyme and eventually inhibits. Pepsin digestion of 3-PGDH treated with p-nitrophenylacetate yielded a peptide, Ala-Ser-CysSAc; this, like the work with iodoacetate, indicates that glutathione is not involved in the inhibition (Cunningham and Schepman, 1963).

3-PGDH isolated from muscle is usually 80–90% in the oxidized state and requires incubation with cysteine or glutathione for activation. In the oxidized, or disulfide, state the enzyme does not react with iodoacetate, and this may be responsible for some of the deviations in susceptibility reported. For example, Chefurka (1954) found 3-PGDH from flies to be more resistant to iodoacetate than the enzyme from rabbit muscle, no inhibition being observed after 30 min at pH 7.1 with 0.09 m M iodoacetate, but here it is difficult to determine the state of the enzyme during incubation. If 3-PGDH is incubated with iodoacetate and then an activator added, it appears that little inhibition occurs.
It was first observed by Green et al. (1937 b) that the presence of a substrate (in this case glyceraldehyde), can protect 3-PGDH against inactivation by iodoacetate, and this was confirmed for the pure enzyme and the natural substrate. 3-P-glyceraldehyde, by Holzer and Holzer (1962), who pointed out that the ability of a substrate to protect is inversely proportional to the $K_m$, as would be expected. Substrate protection has also been recorded by Boyer and Segal (1953), Segal and Boyer (1953), and Krimsky and Racker (1954). The following do not protect against iodoacetate: NAD, phosphate, and arsenate (which can replace phosphate in the transacylation). The failure of NAD to protect might be taken as evidence that the NAD is not bound tightly to the SH group with which iodoacetate reacts, or is not bound at this group at all. Indeed, Racker and Krimsky (1958) found that the reaction with iodoacetate occurs more rapidly in the presence of NAD than in its absence, possibly indicating that NAD brings about a structural change in the active center. Carboxymethylation of approximately 5 SH groups of 3-PGDH is required for complete inactivation and, of these, 2 groups can be specifically protected by substrate (Boyer and Segal, 1954). An SH group not involved in binding of NAD can be protected by the substrate, since protection occurs in both the absence and presence of bound NAD. Thus it may be that the combination of the enzyme with the substrate does not involve an aldehydolysis of an $S$—NAD bond but a direct reaction with another SH group. Since various phosphorylated substances which are not aldehydes (e.g., P-glycerate) can protect the enzyme to some extent, the formation of a thiohemiacetal link is not necessary, and it was postulated that a second point of attachment for iodoacetate more related to the binding of phosphate may be assumed (Krimsky and Racker, 1954). However, as pointed out many times previously, protection by a substance does not necessarily provide evidence of the groups with which either the inhibitor or the protector reacts.

A number of reactions are catalyzed by 3-PGDH under various conditions and the effects of iodoacetate on these are often puzzling. The oxidation of NADH by acetyl-P is well inhibited by iodoacetate, as expected, since this is essentially the reverse of the normal reaction (Racker and Krimsky, 1952). The oxidation of NADH by various dyes (a diaphorase-like action) is also inhibited (Rafter and Colowick, 1957). However, the hydrolysis of acetyl-P is not only not inhibited by 5 mM iodoacetate (Park and Koshland, 1958), but may under certain conditions be accelerated by treatment with iodoacetate (Krimsky and Racker, 1955). This has been explained by assuming that an alteration of the structure of the active center is required for this hydrolytic activity, so that the usual acyl-enzyme complex is formed but the reaction is diverted. Arsenolysis of acetyl-P is inhibited by iodoacetate but not as readily as is the oxidation of 3-P-glyceraldehyde (Racker and Krimsky, 1952; Kaplan et al., 1957); 1–2 mM iodoacetate may inhibit 25–
75% in most cases. The arselenolysis and certainly the hydrolysis of acetyl-P must not occur through reaction with the SH group attacked primarily by iodoacetate. On the other hand, the hydrolysis of p-nitrophenylacetate is quite readily inhibited (Park et al., 1961). This substrate is also not arselenolyzed, so the mechanism of the reaction must be different from that of acetyl-P.

**Alcohol and Lactate Dehydrogenases**

The formation of ethanol or lactate in anaerobic glycolysis depends on the alcohol and lactate dehydrogenases, respectively, through a coupled oxidoreduction with 3-PGDH, and thus it is of some importance to determine as far as possible if the inhibition of glycolysis by iodoacetate is due in part to effects on these enzymes. We have already seen that Dixon (1937) found yeast alcohol dehydrogenase to be the most sensitive enzyme he tested and believed it to be the site of inhibition of yeast fermentation, whereas lactate dehydrogenase is scarcely inhibited so that the depression of muscle glycolysis cannot be attributed to a block of this enzyme. There has been some variability in the subsequent reports on the sensitivity of alcohol dehydrogenase to iodoacetate (Table 1-4); however, it has generally been confirmed that the yeast enzyme is fairly susceptible, although perhaps not as much as originally reported by Dixon. A rather odd fact is that the most recent work with the purest enzyme has indicated the least sensitivity. This makes it very difficult to evaluate the role of alcohol dehydrogenase in iodoacetate inhibition: the results of Dixon certainly suggest that in yeast the inhibition of fermentation must to a great extent be attributed to an effect on this enzyme as well as 3-PGDH, and the data of Mackworth (1948) generally confirm this, but the results of Barron and Levine (1952) point to only a minor role of alcohol dehydrogenase in the inhibition. Comparable concentration-inhibition curves for each enzyme are lacking. The work of Aldous (1952) is interesting because he incubated yeast with 1.5 mM iodoacetate for 30 min and then extracted the enzymes, finding 83% inhibition of the alcohol dehydrogenase. The trouble here is that the pH was 4.5 to facilitate penetration of the iodoacetate and glycolysis was blocked completely; thus one cannot compare the effects on 3-PGDH and alcohol dehydrogenase, nor can one use these results to estimate the intracellular inhibition of alcohol dehydrogenase at other pHs. It may be noted that pyruvate decarboxylase is inhibited 87%, lactate dehydrogenase 94%, and even catalase 90%, so that under these conditions no specificity of any kind was achieved.

Lutwak-Mann (1938) emphasized that horse liver alcohol dehydrogenase is much less sensitive to iodoacetate than the yeast enzyme. This is not due to inactivation of the iodoacetate by the liver preparation, since incubation of minced horse liver with iodoacetate (little inhibition produced) and then testing this on the yeast enzyme led to marked inhibition. Inasmuch as so
### Table 1-4

**Inhibition of Alcohol Dehydrogenase by Iodoacetate**

<table>
<thead>
<tr>
<th>Source of enzyme&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Iodoacetate (mM)</th>
<th>Preincubation (min)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acetobacter sp.</em>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3</td>
<td>—</td>
<td>0</td>
<td>Nakayama (1961 b)</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>1</td>
<td>—</td>
<td>0</td>
<td>Azoulay and Heydeman (1963)</td>
</tr>
<tr>
<td><em>Leuconostoc mesenteroides</em>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10</td>
<td>30</td>
<td>100</td>
<td>DeMoss (1954)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>30</td>
<td>58</td>
<td></td>
</tr>
<tr>
<td>Yeast</td>
<td>0.33</td>
<td>10</td>
<td>92</td>
<td>Dixon (1937)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>10</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.33</td>
<td>15</td>
<td>60</td>
<td>Mackworth (1948)</td>
</tr>
<tr>
<td>Yeast (<em>in vivo</em>)</td>
<td>1.5</td>
<td>30</td>
<td>83</td>
<td>Aldous (1952)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10</td>
<td>30</td>
<td>Barron and Levine (1952)</td>
</tr>
<tr>
<td><em>Lilium aurantum</em> pollen</td>
<td>1</td>
<td>—</td>
<td>64</td>
<td>Okunuki (1940)</td>
</tr>
<tr>
<td><em>Avena sativum</em> (oats)</td>
<td>0.01</td>
<td>3</td>
<td>14</td>
<td>Berger and Avery (1943)</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>3</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>3</td>
<td>&gt;98</td>
<td></td>
</tr>
<tr>
<td>Wheat germ (NAD and NADP)</td>
<td>1</td>
<td>10</td>
<td>50</td>
<td>Stafford and Vennesland (1953)</td>
</tr>
<tr>
<td><em>Oryza sativa</em> (rice)</td>
<td>1</td>
<td>60</td>
<td>20</td>
<td>App and Meiss (1958)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>120</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>60</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Rat liver</td>
<td>0.23</td>
<td>5</td>
<td>5</td>
<td>Adler <em>et al.</em> (1938)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Horse liver</td>
<td>10</td>
<td>7</td>
<td>14</td>
<td>Lutwak-Mann (1938)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>7</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>—</td>
<td>0</td>
<td>Bonnichsen and Brink (1955)</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>—</td>
<td>0</td>
<td>Snyder <em>et al.</em> (1963)</td>
</tr>
</tbody>
</table>

<sup>a</sup> The enzymes are all NAD-dependent unless otherwise noted.

<sup>b</sup> This enzyme is a new type of alcohol dehydrogenase, perhaps better called an alcohol:cytochrome-553 reductase.

<sup>c</sup> It was stated by DeMoss (1955) that this enzyme is inhibited 100% by 1 mM iodoacetate after 7 min incubation with respect to NADP reduction; the discrepancy between these figures and those in the table is not explained.
limited a variety of sources for alcohol dehydrogenase has been examined, it is impossible to draw conclusions about the sensitivity of the enzyme throughout the plant and animal kingdoms, but there is no doubt that the plant enzyme is reasonably easily inhibited. One must conclude, at least in most work where iodoacetate has been used in concentrations greater than are required to block 3-PGDH effectively, that the alcohol dehydrogenase must be inhibited to some extent.

Little is known about the detailed mechanism of the inhibition. The yeast alcohol dehydrogenase is protected from iodoacetate by both ethanol and NAD (Barron and Levine, 1952), indicating the inhibition to be dynamically competitive, and the SH groups attacked to be at or near the active center. It is also possible that some of the inhibition observed with iodoacetamide may not be entirely related to carboxymethylation of SH groups, since Woronick (1961) found various acetamides to inhibit horse liver alcohol dehydrogenase competitively with respect to acetaldehyde, forming complexes of the EI and E-NAD-I types (see accompanying tabulation). Yet

<table>
<thead>
<tr>
<th>Amide</th>
<th>Competitive $K_s$ (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetamide</td>
<td>10.5</td>
</tr>
<tr>
<td>Fluoroacetamide</td>
<td>31.4</td>
</tr>
<tr>
<td>Chloroacetamide</td>
<td>6.29</td>
</tr>
<tr>
<td>Bromoacetamide</td>
<td>3.86</td>
</tr>
<tr>
<td>Iodoacetamide</td>
<td>1.83</td>
</tr>
<tr>
<td>Propionamide</td>
<td>0.44</td>
</tr>
</tbody>
</table>

McKinley-McKee (1963) claim that neither alcohol nor acetaldehyde protects horse liver alcohol dehydrogenase against iodoacetamide. On the other hand, NADH protects 2 SH groups per mole of enzyme from attack by 36 mM iodoacetate, and the loss of enzyme activity can be attributed to the carboxymethylation of these SH groups when the enzyme is not complexed with NADH (Li and Vallee, 1963). Treatment of the enzyme with iodoacetate-1-C$^{14}$ followed by trypsin digestion allowed the isolation of a single labeled polypeptide of the structure:

Met—Val—Ala—Thr—Gly—Ileu—Cys—Arg

\[
\begin{array}{c}
\text{CH}_2 \\
\text{COO}^- \\
\end{array}
\]

suggested that the apodehydrogenases contains two identical chains (Li and Vallee, 1964). Zn$^{++}$ is necessary for the activity but this peptide does not bind Zn$^{++}$, so this ion must be attached to an adjacent chain.
The evidence for a reasonably selective action of iodoacetate on muscle 3-PGDH relative to lactate dehydrogenase is much better. First, lactate dehydrogenases from most sources are not readily inhibited (Table 1-5),

<table>
<thead>
<tr>
<th>Source of enzyme</th>
<th>Iodoacetate (mM)</th>
<th>Preincubation (min)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionibacterium pentosaceum</td>
<td>6</td>
<td>2</td>
<td>26</td>
<td>Molinari and Lara (1960)</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>8.2</td>
<td>2</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Tetrahymena pyriformis</td>
<td>4</td>
<td>—</td>
<td>0</td>
<td>Yamamura et al. (1952)</td>
</tr>
<tr>
<td>Yeast</td>
<td>0.33</td>
<td>10</td>
<td>0</td>
<td>Eichel and Rem (1962)</td>
</tr>
<tr>
<td>Yeast (in vivo)</td>
<td>10</td>
<td>10</td>
<td>7</td>
<td>Dixon (1937)</td>
</tr>
<tr>
<td>Chick embryo liver</td>
<td>1.5</td>
<td>30</td>
<td>94</td>
<td>Yamashita (1960)</td>
</tr>
<tr>
<td>Chick embryo homogenate</td>
<td>30</td>
<td>10</td>
<td>0</td>
<td>Solomon (1958)</td>
</tr>
<tr>
<td>Pigeon muscle</td>
<td>30</td>
<td>10</td>
<td>0</td>
<td>Solomon (1958)</td>
</tr>
<tr>
<td>Rabbit muscle</td>
<td>167</td>
<td>—</td>
<td>100</td>
<td>Das (1937 a)</td>
</tr>
<tr>
<td>Rat muscle</td>
<td>33</td>
<td>10</td>
<td>0</td>
<td>Dixon (1937)</td>
</tr>
<tr>
<td>Ox heart</td>
<td>1</td>
<td>10</td>
<td>62</td>
<td>Hopkins et al. (1938)</td>
</tr>
<tr>
<td>Pig heart</td>
<td>13.3</td>
<td>20</td>
<td>&lt;5</td>
<td>Dube et al. (1963)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>5</td>
<td>5</td>
<td>Adler et al. (1938)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>5</td>
<td>30</td>
<td>Neillands (1954)</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>5</td>
<td>80</td>
<td>Green and Brostieux (1936)</td>
</tr>
</tbody>
</table>

Table 1-5
INHIBITIONS OF LACTATE DEHYDROGENASE BY IODOACETATE

\[a\] These enzymes were called lactate oxidases.
\[b\] Iodoacetamide.

and in no case has the enzyme been found to be nearly as sensitive as 3-PGDH. The lactate dehydrogenase from beef heart contains 7–9 SH groups, but only 3 of these react with p-mercuribenzoate in the native state and none reacts with iodoacetate under the usual experimental conditions (Ny-
gaard, 1956; Pfeiderer et al., 1958). Rabbit muscle lactate dehydrogenase is more readily inhibited by iodoacetamide than iodoacetate, and yet rather high concentrations and relatively long incubations (2.5 hr at 37°) are required to inhibit significantly (Dube et al., 1963). The enzyme is protected by NAD and oxalate together, but not by either alone, and it was suggested that a sluggish SH group occurs at the active site, although possibly other groups are involved. Thus it is likely that the inhibition of muscle glycolysis by reasonable concentrations of iodoacetate is not due at all to effects on the lactate dehydrogenase. We shall see, furthermore, that the addition of lactate to iodoacetate-treated tissues usually induces a good O₂ uptake, indicating that the dehydrogenase cannot be very significantly inhibited.

**Succinate Dehydrogenase**

Since the earlier work of Hopkins and Morgan (1938) showing the inhibition of succinate dehydrogenase by iodoacetate and a variety of SH reagents, this enzyme has been one of the classic SH enzymes and has probably been studied more in this regard than any other. In this study the methylene blue reduction technique was used and thus iodoacetate must act on the dehydrogenase rather than the rest of the electron transport sequence. The only other possible site in the sequence is just before the cytochromes. The only data indicating an effect elsewhere than on the dehydrogenase is that of Miller (1951), who found potato succinate oxidase to be inhibited weakly by iodoacetate but succinate dehydrogenase not at all; since similar results were found with malonate, one must assume the operation of unknown factors. Some inhibitions are presented in Table 1-6.

Succinate dehydrogenase must be classed as a moderately sensitive enzyme with respect to iodoacetate inhibition, and it is by no means as inhibitable as 3-PGDH. However, in some cases it appears that significant inhibition would result from the use of iodoacetate at concentrations around 1 mM. It is not the most sensitive enzyme of the cycle (Yang, 1957) but at 1 mM it would be strongly inhibited, and these results are quite comparable with those obtained on preparations from other mammalian tissues. One characteristic of the enzyme is the sluggishness with which the groups react with iodoacetate. Despite the fact that Hopkins and Morgan showed clearly that the reaction is quite slow, very few investigators have preincubated the enzyme before examination of the activity and fewer have mentioned whether preincubation was carried out or not, as can be seen from the table. Results with inhibitors such as iodoacetate are quite meaningless unless the time relations are considered. In long-term experiments particularly, one must be careful in using data from isolated enzyme preparations, since after several hours the inhibition on succinate dehydrogenase may be much more than is indicated. Of course, the sensitivity of the enzyme in the cell may be different from that in the extracted state. Another characteristic is the
<table>
<thead>
<tr>
<th>Source of enzyme</th>
<th>Iodoacetate (mM)</th>
<th>Preincubation (min)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterium tularense</td>
<td>3.5</td>
<td>—</td>
<td>100</td>
<td>Wadkins and Mills (1955)</td>
</tr>
<tr>
<td>Micrococcus lactilyticus</td>
<td>45(\textsuperscript{b} )</td>
<td>—</td>
<td>34</td>
<td>Warringa and Giuditta (1958)</td>
</tr>
<tr>
<td>Rhizobium</td>
<td>1</td>
<td>—</td>
<td>63</td>
<td>Burris and Wilson (1939)</td>
</tr>
<tr>
<td>10</td>
<td>—</td>
<td></td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Penicillium chrysogenum</td>
<td>9</td>
<td>—</td>
<td>100</td>
<td>Godzeski and Stone (1955)</td>
</tr>
<tr>
<td>Trypanosoma cruzi</td>
<td>0.2(\textsuperscript{b} )</td>
<td>—</td>
<td>13</td>
<td>Agosin and von Brand (1955)</td>
</tr>
<tr>
<td>2</td>
<td>—</td>
<td></td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>—</td>
<td></td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Avena coleoptile</td>
<td>0.144</td>
<td>—</td>
<td>58</td>
<td>Bonner (1949)</td>
</tr>
<tr>
<td>0.48</td>
<td>—</td>
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<td>100</td>
<td></td>
</tr>
<tr>
<td>Potato tubers</td>
<td>0.1</td>
<td>—</td>
<td>6</td>
<td>Millerd (1951)</td>
</tr>
<tr>
<td>1</td>
<td>—</td>
<td></td>
<td>7</td>
<td></td>
</tr>
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<td>20</td>
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</tr>
<tr>
<td>Oyster eggs</td>
<td>1</td>
<td>—</td>
<td>12</td>
<td>Cleland (1949)</td>
</tr>
<tr>
<td>10</td>
<td>—</td>
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<td>Oyster muscle</td>
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<td>—</td>
<td>39</td>
<td>Humphrey (1947)</td>
</tr>
<tr>
<td>10</td>
<td>—</td>
<td></td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>—</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Aedes aegypti (mosquito)</td>
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<td>—</td>
<td>0</td>
<td>Gonda et al. (1957)</td>
</tr>
<tr>
<td>1</td>
<td>—</td>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>0.1(\textsuperscript{b} )</td>
<td>—</td>
<td></td>
<td>0</td>
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<tr>
<td>Lucilia cuprina (blowfly) midgut</td>
<td>50</td>
<td>—</td>
<td>100</td>
<td>Waterhouse and Stay (1955)</td>
</tr>
<tr>
<td>Pigeon muscle</td>
<td>1</td>
<td>—</td>
<td>42</td>
<td>Barron and Singer (1945)</td>
</tr>
<tr>
<td>5(\textsuperscript{b} )</td>
<td>—</td>
<td></td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Rabbit muscle</td>
<td>0.33</td>
<td>10</td>
<td>0</td>
<td>Dixon (1937)</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
<td></td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>60</td>
<td></td>
<td>90</td>
<td>Hopkins et al. (1938)</td>
</tr>
<tr>
<td>Pig heart</td>
<td>10</td>
<td>10</td>
<td>43</td>
<td>Hopkins and Morgan (1938)</td>
</tr>
<tr>
<td>8.2</td>
<td>30</td>
<td></td>
<td>90</td>
<td>Stoppani and Brignone (1956)</td>
</tr>
<tr>
<td>12</td>
<td>—</td>
<td></td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>10.8</td>
<td>—</td>
<td></td>
<td>67</td>
<td>Stoppani and Brignone (1957)</td>
</tr>
</tbody>
</table>
### Table 1-6 (continued)

<table>
<thead>
<tr>
<th>Source of enzyme</th>
<th>Iodoacetate (mM)</th>
<th>Preincubation (min)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ox heart</td>
<td>$1^b$</td>
<td>—</td>
<td>71</td>
<td>Singer et al. (1956 b)</td>
</tr>
<tr>
<td>Rat heart</td>
<td>0.01</td>
<td>10</td>
<td>4</td>
<td>Yang (1957)</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>10</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>10</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>Rat liver</td>
<td>1</td>
<td>0</td>
<td>10</td>
<td>Potter and DuBois (1943)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>20</td>
<td>52</td>
<td>Hirade and Hayashi (1953)</td>
</tr>
<tr>
<td></td>
<td>0.17$^b$</td>
<td>—</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.7</td>
<td>—</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>—</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>170</td>
<td>—</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>—</td>
<td>100</td>
<td>Wadkins and Mills (1955)</td>
</tr>
<tr>
<td>Rat kidney</td>
<td>9</td>
<td>—</td>
<td>54</td>
<td>Hirade (1952)</td>
</tr>
<tr>
<td>Mouse kidney</td>
<td>2</td>
<td>20</td>
<td>48</td>
<td>Ames et al. (1946)</td>
</tr>
</tbody>
</table>

---

$a$ The activities were determined in several different ways: in some the dehydrogenase was measured directly with a dye, in others the oxygen uptake from succinate in either crude preparations or mitochondria, with or without added electron transporters. Since the dehydrogenase has been shown to be the sensitive portion of the total oxidase, it is likely that the results refer to inhibition of the dehydrogenase only.

$b$ Iodoacetamide used rather than iodoacetate.

rather small change in inhibition as the iodoacetate is increased; this may well be a reflection of the sluggishness of the reactions. From inspection of the table, succinate dehydrogenase from all forms of life seems to be of approximately equal sensitivity to iodoacetate, although mammalian enzymes may be somewhat more easily inhibited.

There is very little work on the mechanism of the inhibition or on the enzyme groups reacted. When inhibition proceeds so slowly, one must be more concerned with reaction of groups other than SH. Barron and Singer (1945) provided evidence that non-SH groups may be involved in pigeon muscle succinate dehydrogenase. The enzyme was allowed to react with $p$-chloromercuribenzoate to complete inhibition; some of this enzyme was then incubated with either iodoacetate or iodoacetamide for 35 min. Reactivation with glutathione gave 84% recovery of the enzyme treated with only the mercurial, 68% recovery when treated with mercurial and iodoacetamide, and 13% recovery when treated with mercurial and iodoacetate. If the mercurial is assumed to react with SH groups only, they reasoned that iodoacetate must react with something else. However, there are several
other explanations. It is possible, although perhaps not likely, that iodoacetate replaces some of the mercurial from the SH groups; since glutathione reactivates quite rapidly, the mercurial cannot be very firmly attached. Also the mercurial might not have reacted with all the SH groups on the enzyme, although enough to inhibit the activity, so that iodoacetate by reacting with the remaining groups could alter the enzyme so that it had lost some activity even when the mercurial was removed. Finally, it may be that excess iodoacetate present reacted with the glutathione, thereby reducing its ability to reactivate the enzyme. Hirade and Hayashi (1953) found disappearance of SH groups and inhibition by iodoacetamide to proceed in parallel fashion with rat liver enzyme, but for some reason obtained only 21% inhibition and 11% of the SH groups reacted with 170 mM inhibitor; they do not state how long the reaction ran.

Ribonuclease

Zittle (1946) found that pancreatic ribonuclease is inhibited slowly and moderately by iodoacetate and iodoacetamide, and felt that this probably indicated the presence of SH groups. However, he was troubled by the fact that iodoacetate inhibits faster and better than iodoacetamide, contrary to the usual experience with other enzymes, but felt that other explanations must be tested before assuming that groups other than SH are reacted. We now know that ribonuclease possesses no SH groups. A number of workers have found iodoacetate to have little or no action on ribonucleases: 2–10 mM on pancreatic enzyme (Davis and Allen, 1955; Rabinovitch and Barron, 1955; Dickman et al., 1956), 100 mM on Bothrops venom enzyme (Taborda et al., 1952 a,b), up to 30 mM on the tobacco leaf enzyme (Frisch-Niggemeyer and Reddi, 1957), and up to 48 mM on Bacillus subtilis enzyme (Watanabe and Yamafuji, 1961). Indeed, several workers have observed a stimulation of the activity: thus the ribonuclease in Taka-diastase is stimulated 20% by 10 mM iodoacetate (Naoi-Tada et al., 1959). The enzyme from ascites tumor cells is first stimulated at low concentrations, reaching a peak near 0.001 mM, and is then depressed (Fig. 1-1) (Hilz and Klempien, 1959). Other SH reagents may also stimulate. Roth (1956, 1958) believed the stimulation might arise from the inactivation by the SH reagent of a naturally occurring inhibitor present in unpurified preparations. It appears in any event that, under the usual conditions, iodoacetate has very little effect on ribonuclease, and probably would not inhibit it in cellular preparations at the concentrations used to block glycolysis. The interest in the effects of iodoacetate on ribonuclease come from studies done not under the usual physiological conditions but at relatively high iodoacetate concentrations and over a wide range of pH. When 30 mg of ribonuclease and 30 mg of iodoacetic acid in 10 ml are allowed to react at 40°, and amino acid analyses of the enzyme are then made, it is found that
the amino acids reacted are quite different in the three pH ranges used (Gundlach et al., 1959 a). Reaction at pH 5.5–6.0 leads to a chromatographically homogeneous protein which differs from the initial ribonuclease only in the carboxymethylation of a single imidazole group of histidine. At pH 2.8 a single methionine and at pH 8.5 a single lysine react with iodoacetate. The rate of inactivation of ribonuclease also depends on the pH:

![Graph showing activation and inhibition of ascites tumor ribonuclease by iodoacetate and p-mercuribenzoate.](image)

At pH 8.5–10.5 it is slow (half-reaction in 90 min and maximal inhibition 65%), at pH 5.5–6.2 it is faster and more complete (maximal inhibition 90%), at pH 4 it is again slow, and at pH 2.8 it is more rapid. This is well explained on the basis of different amino acids reacting at the different pH’s. The inactivation with bromoacetate behaves similarly (see accompanying tabulation) (Barnard and Stein, 1959). Only one of the four histi-

<table>
<thead>
<tr>
<th>pH</th>
<th>Time for 50% inhibition (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2</td>
<td>140</td>
</tr>
<tr>
<td>5.3</td>
<td>14</td>
</tr>
<tr>
<td>7.1</td>
<td>27</td>
</tr>
<tr>
<td>9.0</td>
<td>46</td>
</tr>
</tbody>
</table>
dine residues in ribonuclease is carboxymethylated and the rate is much greater than for free histidine, indicating that this single histidine must be in a particularly reactive state. The reactive histidine is the one nearest the C-terminal end of the protein chain (position 119) (Stein and Barnard, 1959). Two chromatographically distinct monooxycarbonylmethylated derivatives are found when ribonuclease A is treated with iodoacetate at pH 5.5 (Crestfield et al., 1963). The major derivative is 1-carboxymethylhistidine-119-ribonuclease, i.e., with the histidine in position 119 carboxymethylated at the 1-position of the imidazole ring, and the minor product is 3-carboxymethylhistidine-12-ribonuclease. Thus histidine residues in two different regions of the enzyme are attacked at different sites on the imidazole ring. The major product is inactive while the minor one is active. The variation of the alkylation rate with pH has been treated satisfactorily by Lamden et al. (1962) on the simple assumption that iodoacetate reacts with only the singly ionized form of the enzyme, i.e., with EH in the equilibrium $\text{EH}_2 \rightleftharpoons \text{EH} \rightleftharpoons \text{E}$. The $pK_a$'s for the enzyme groups were calculated to be 4.85 and 5.55, which are quite low for imidazole, so they raise some doubt whether the inhibition can be immediately related to reaction with the histidine residues. Various treatments of ribonuclease — oxidation by performate, denaturation with urea or guanidine — abolish the reactivity of histidine with iodoacetate (Stark et al., 1961). Furthermore, iodoacetamide does not react with this histidine at pH 5.5 and 40°, a marked difference from iodoacetate; since both react with $\alpha$-N-acetyl-L-histidine at the same rate, the difference in the enzyme must be due to the environment of the histidine residue. The three-dimensional structure of the enzyme is necessary for the activation of the histidine residue, and a positive charge was postulated to be located nearby. Ribonuclease-S (subtilisin-modified ribonuclease) consists of a protein portion and a 20-residue peptide. Inactivation of the protein portion by iodoacetate at pH 6 is caused by the carboxymethylation of methionine residues, resulting in the inability to bind the peptide and form an active enzyme (Vithayathil and Richards, 1961). No reaction with histidine residues occurs under these conditions. However, the total enzyme at pH 6 is reacted at a histidine residue on the protein portion so that the peptide component is necessary for the reactivity of this residue. One is thus led to conclude from these recent studies that iodoacetate can react with amino acid residues other than cysteine, although the rates are slow unless very high concentrations of iodoacetate are used, but, more important, that the reactivity of a particular amino acid depends strongly on the steric and electrostatic factors arising from the environment on the enzyme surface. These results have introduced new methods for the use of iodoacetate in studying enzyme mechanisms.
Adenosinetriphosphatase and Related Enzymes

The behavior of ATPase with respect to iodoacetate becomes important in considering the effects of iodoacetate on muscle. ATPase is classed as an SH enzyme due to its inhibition primarily by mercurials. However, it appears to be one of the enzymes whose SH groups are relatively unreactive to iodoacetate. Needham (1942) reported that myosin ATPase is not inhibited by 3 mM iodoacetate even after 2 hr incubation, although all of the SH groups giving the nitroprusside test are reacted, and Singer and Barron (1944) found no inhibition on reaction of myosin ATPase with 3 mM iodoacetamide. Engelhardt and Lyubimova (1942) observed a 30% inhibition with 10 mM iodoacetate, and Engelhardt (1946) remarked on the strange unreactivity of myosin SH groups with iodoacetate. The results of Polis and Meyerhof (1947) were very erratic, but they found in general some stimulation at low concentrations (below 1 mM) of iodoacetate and iodoacetamide, and small inhibitions at higher concentrations, the response apparently depending on the state of the myosin. Bailey and Perry (1947) observed a very slow reaction of myosin with iodoacetamide, some 15 hr being required for completion at 16° and concentrations between 1.7 and 42 mM. A similar sluggishness was noted by Bárány and Bárány (1959 a), iodoacetate at 100 mM and pH 8 reducing the SH groups by one third in 2 hr, and iodoacetamide reacting about twice as rapidly, the slower reaction with iodoacetate being attributed to the negative charge on the enzyme. In contrast to the early work of Needham, they found that myosin loses most of its ATPase activity before half of its SH groups are reacted. There are at least two different types of SH group in myosin. Of the 15 SH groups (per molecular weight unit of 200,000), only 14 can exchange with the disulfide reagent, bis-(β-carboxyethyl)disulfide; if the myosin treated with this substance is then exposed to iodoacetamide, the activity is not restored by β-mercaptoethanol, as it is in the absence of iodoacetamide (Stracher, 1963). Thus there seems to be one SH group at the active site, and this was confirmed by incubating myosin with labeled iodoacetamide and isolating S-carboxymethylcysteine. It is difficult to generalize on the behavior of myosin ATPase in the presence of these inhibitors, but it seems safe to say that there will be little if any inhibition at the concentrations used to produce the characteristic changes in muscle contractility, unless the susceptibility of myosin ATPase is greater in vivo.

The results with the ATPases from other sources are equally variable, due perhaps to the different types of ATPase activity measured. In general, rather small inhibitions have been reported: 29% by 2 mM iodoacetate for the guinea pig brain enzyme (Gore, 1951), 40% by 10 mM iodoacetate for insect muscle enzyme (Gilmour, 1955), 30% by 10 mM iodoacetate for human erythrocyte enzyme (Herbert, 1956), and 17% by 100 mM iodoacetate for the potato apyrase (Bárány and Bárány, 1959 b). Mitochondrial
ATPase is either not affected (Pullman et al., 1960) or stimulated (Charibitron and Avi-Dor, 1959; Fanestil et al., 1963). Such effects may not be directly on the ATPase, since any agent that prevents the mitochondrial swelling produced by iodoacetate counteracts the stimulation. Again we conclude that at concentrations blocking glycolysis little effect on the ATPases should be observed, and that modifications in tissue function brought about by iodoacetate probably do not relate to inhibition of this enzyme. Certain ATPases believed to be involved in cellular activities have been examined recently and none is very sensitive: the ATPase in Mytilus spermatozoa tails is inhibited 13% by 0.1 mM iodoacetate (Nelson, 1959), that in the eel electric organ is depressed only 3% by 1 mM iodoacetamide (Glynn, 1963), that in the cortex of sea urchin eggs, which is K⁺-Na⁺-activated and believed to participate in active transport, is inhibited 23% by 1 mM iodoacetate (Ohnishi, 1963), and the ATPase in rabbit kidney which may play a role in Na⁺ resorption is not affected by 1 mM iodoacetate or 10 mM iodoacetamide (Taylor, 1963). These enzymes will be affected if high concentrations of the inhibitors are used, but all the evidence indicates their inhibition not to be significant in the range used to depress glycolysis.

The exchange reactions ADP-ATP or Pᵢ-ATP catalyzed by mitochondrial or chloroplast systems are inhibited by iodoacetate only at quite high concentrations (around 10 mM) (Plant, 1957; Kahn and Jagendorf, 1961; Chiga and Plant, 1959). The AMP-ATP transphosphorylase (myokinase) of rabbit muscle is not inhibited by 1 mM iodoacetate without preincubation (Noda, 1958), although 2 SH groups are free for reaction with certain other SH reagents. A thorough kinetic study of this inhibition has been made by Mahowald et al. (1962), who found the rate of reaction and degree of inhibition to be low unless fairly high ratios of iodoacetate to enzyme were used. The reactions were run at pH 8 and 30⁰ and yet at a ratio of (I)/(E) = 0.48 (iodoacetate 0.27 mM) it requires 3 hr for maximal reaction, at which time the inhibition is only 25%, and even at a ratio of (I)/(E) = 2.88 (iodoacetate 1.61 mM) it requires several hours for the reaction to be completed, although here the inhibition is complete. The inactivated enzyme was found to contain 1.83 S-carboxymethylcysteine residues with 0.2 half-cystine residue remaining. Thus 2 SH groups per enzyme molecule react with iodoacetate but the rate is so slow that the enzyme is unlikely to be significantly inhibited in vivo.

**Creatine Kinase (ATP:Creatine Phosphotransferase)**

Although the phosphate-splitting and -transferring enzymes in general are not readily inhibited by iodoacetate, one enzyme quite important in determining the maintenance of tissue function, namely, creatine kinase, is very sensitive to iodoacetate, at least in the isolated state (Table 1–7). We shall discuss later the possible inhibition of this enzyme in muscles
### Table 1-7

**Inhibition of Creatine Kinase by Iodoacetate**

<table>
<thead>
<tr>
<th>Source of enzyme</th>
<th>Iodoacetate (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frog muscle</td>
<td>0.5</td>
<td>88</td>
<td>Carlson and Siger (1959)</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>35</td>
<td>Padieu and Mommaerts (1960)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Rat muscle</td>
<td>0.1</td>
<td>13</td>
<td>Cori et al. (1958)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Sheep muscle</td>
<td>0.001</td>
<td>12</td>
<td>Ennor and Rosenberg (1954)</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Guinea pig brain</td>
<td>0.2</td>
<td>57</td>
<td>Narayanaswami (1952)</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Rabbit erythrocytes</td>
<td>0.01</td>
<td>0</td>
<td>Solvonuk et al. (1956)</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

(page 205). All investigators, despite variation in results, agree that this enzyme in the isolated state is inhibited by very low concentrations of iodoacetate and is usually as sensitive as 3-PGDH, with the exception of Cori et al. (1958). The poor inhibitions observed by the latter workers may possibly be attributed to their incubation of the enzyme and inhibitor at 1º for only 20 min, or to their dialysis of the inhibitor from the enzyme before assaying the activity, or it may be that rat muscle creatine kinase is less reactive than the enzymes from other sources. We may also note that Geiger (1956) reported the creatine kinase from chicken gizzard to be rapidly inactivated by iodoacetate, although no data were given. The corresponding arginine kinase from crayfish muscle may not be as sensitive, inasmuch as Morrison et al. (1957) found only 19% inhibition at 1 mM iodoacetate and 92% at 10 mM iodoacetate. Treatment of muscle with iodoacetate followed by extraction and examination of 3-PGDH and creatine kinase showed that the former enzyme could be inhibited completely without affecting the kinase at all, which is probably explained by the protection provided by a variety of substances (creatine-P, ATP, ADP, etc.) (Padieu and Mommaerts, 1960). Carlson and Siger (1959) also found the creatine kinase to be less readily inhibited in muscle, and that treatment of muscle with 0.5 mM iodoacetate for 30 min at 20º blocks glycolysis without significantly affecting the kinase. One must thus conclude that despite the
high sensitivity of this enzyme when isolated, the situation may be different in intact muscle, and the effects of iodoacetate in vivo are likely to depend on a variety of factors.

The alkylation of creatine kinase by iodoacetate and iodoacetamide is not altered by variation of the pH from 6 to 9 but is sensitive to changes in the ionic strength (Fig. 1-2) (Rabin and Watts, 1960; Watts, 1963; Watts et al., 1960). Alkylation of a single SH group is sufficient to inactivate the enzyme and possibly a positive charge near this group is responsible for the ionic strength effect with iodoacetate. The reaction with iodoacetamide is not affected by ionic strength, proving that it is not just a configurational change of the enzyme (Watts and Rabin, 1962). The enzyme is protected against iodoacetate by creatine-P and ATP as long as Mg$^{++}$ is present; neither of the substrates alone nor Mg$^{++}$ protects, indicating a Mg-complex as the true substrate. The SH group at the active site is pictured as lying adjacent to a histidine residue, with which it forms a hydrogen bond, this explaining to some extent the lack of pH effects. Using an iodide electrode to determine the release of iodide during reaction of the SH groups, the rate of inactivation was found to parallel the rate of carboxymethylation by iodoacetate (Fig. 1-3) (Watts et al., 1961). It may well be that creatine kinase can exist in different forms, inasmuch as Watts et al. (1962) found that substrates protected only one SH group in one sample but protected both of the SH groups in another. Another complication is introduced by the observation of Mahowald et al. (1962) that reaction of the kinase with
1.5 mM iodoacetate at pH 8.8 and 0° for 30 min leads to some alkylation of methionine, histidine, and lysine. However, 2 SH groups are also reacted and 5.7 half-cystine residues remain. The susceptible SH group may be linked under certain conditions to an imidazole ring through a hydrogen bond, and this may alter the reactivity of the SH group with alkylating agents (Rabin and Watts, 1960).

![Graph](image_url)

**Fig. 1-3.** Relationship between the reaction of SH groups (determined by iodide release) and inactivation of creatine kinase by 0.5 mM iodoacetate at pH 9 and ionic strength of 0.1. The curve gives the iodide release and the points the inactivation of the enzyme. (From Watts *et al.*, 1961.)

**Proteolytic Enzymes and Peptidases**

Enzymes hydrolyzing proteins and peptides have been frequently studied with respect to their SH group requirements, because some of the earliest isolated enzymes of this type were found to be readily inhibited by SH reagents. It now appears after numerous proteinases have been tested that in general they may be divided into two classes, those which are quite resistant and do not possess reactive SH groups at the active center, and those which are inhibited strongly, often by low concentrations of SH reagents, as may be seen by inspection of Table 1-8. These enzymes do not constitute a homogeneous group and it is not surprising that the suscepti-
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Iodoacetate (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminopeptidase</td>
<td>Mammalian liver, kidney, and pancreas</td>
<td>1</td>
<td>0</td>
<td>Burstone and Folk (1956)</td>
</tr>
<tr>
<td></td>
<td>Calf lens</td>
<td>6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15</td>
<td>Spector (1963)</td>
</tr>
<tr>
<td></td>
<td>Beef lens</td>
<td>1</td>
<td>0</td>
<td>Wolff and Resnik (1963)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Asclepain &lt;i&gt;m&lt;/i&gt;</td>
<td>&lt;i&gt;Asclepias mexicana&lt;/i&gt; (milkweed) latex</td>
<td>10</td>
<td>99</td>
<td>Greenberg and Winnick (1940)</td>
</tr>
<tr>
<td>Bromelain</td>
<td>Pineapple fruit</td>
<td>20</td>
<td>99</td>
<td>Greenberg and Winnick (1940)</td>
</tr>
<tr>
<td>Bromelain I</td>
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<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94</td>
<td>El-Gharbawi and Whitaker (1963)</td>
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<td>Bromelain IV</td>
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<td>Bromelain V</td>
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<td>Carboxypeptidase</td>
<td>Pancreas</td>
<td>10</td>
<td>100</td>
<td>Smith and Hanson (1949)</td>
</tr>
<tr>
<td>Carnosinase</td>
<td>Pig kidney</td>
<td>1</td>
<td>0</td>
<td>Smith (1955)</td>
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<tr>
<td>Caseinase</td>
<td>&lt;i&gt;Bothrops jaraca&lt;/i&gt; venom</td>
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<td>0</td>
<td>Henriques &lt;i&gt;et al.&lt;/i&gt; (1958)</td>
</tr>
<tr>
<td>Enzyme</td>
<td>Source</td>
<td>$K_i$ (mM)</td>
<td>$K_m$ (mM)</td>
<td>Reference</td>
</tr>
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<td>------------------------</td>
<td>----------------------------</td>
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</tr>
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<td>92</td>
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</tr>
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<td>1</td>
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</tr>
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<td>Cod spleen</td>
<td></td>
<td>1*</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Liver at:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 4</td>
<td></td>
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<td>pH 5</td>
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<td>50</td>
<td>Hagen (1957)</td>
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<td>pH 6</td>
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<td>0.0097</td>
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<td></td>
</tr>
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<td>1</td>
<td>93</td>
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<td>Human brain</td>
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<td>3</td>
<td>0</td>
<td>Ansell and Richter (1954 a)</td>
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<tr>
<td>Calf spleen</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 μmoles/mg E</td>
<td></td>
<td></td>
<td>50</td>
<td>Mauer and Greco (1947)</td>
</tr>
<tr>
<td>Calf thymus</td>
<td></td>
<td>25 μmoles/mg E</td>
<td></td>
<td>Mauer and Greco (1947)</td>
</tr>
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<td>Rat lymphosarcoma</td>
<td></td>
<td>5*</td>
<td>6</td>
<td>Mauer and Thompson (1946)</td>
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<td></td>
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<td>Fruton and Mycek (1956)</td>
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<td>Press et al. (1960)</td>
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<td><em>Clostridium histolyticum</em></td>
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<td>Nordwig and Strauch (1963)</td>
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<td>Dipeptidase</td>
<td>Pig kidney</td>
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<td>Traniello and Vescia (1964)</td>
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<td><em>Flavobacterium elastolyticum</em></td>
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<td>Mandl and Cohen (1960)</td>
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<tr>
<td>Ficin</td>
<td>Fig latex</td>
<td>0.9 mole/mole E</td>
<td>100</td>
<td>Liener (1961)</td>
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<td>Source</td>
<td>Iodoacetate (mM)</td>
<td>% Inhibition</td>
<td>Reference</td>
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<td>Gelatinase</td>
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<td>1</td>
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<td>Yeast</td>
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<tr>
<td>Leucine aminopeptidase</td>
<td>Pig kidney</td>
<td>1(^a)</td>
<td>0</td>
<td>Smith and Spackman (1955)</td>
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<td>40</td>
<td>Green <em>et al.</em> (1955)</td>
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<td></td>
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<td></td>
</tr>
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<td>Lysozyme</td>
<td>Papaya latex</td>
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<td>Smith <em>et al.</em> (1955)</td>
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<td><em>Bacillus brevis</em></td>
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<td>Papaya</td>
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<td>22</td>
<td>Bersin and Logemann (1933)</td>
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<td></td>
<td></td>
<td>1</td>
<td>89</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td>0.5</td>
<td>98</td>
<td>Maschmann and Helmert (1933 a)</td>
</tr>
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<td></td>
<td></td>
<td>1</td>
<td>100</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td>0.27</td>
<td>100</td>
<td>Ganapathy and Sastri (1939)</td>
</tr>
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<td></td>
<td></td>
<td>0.5(^a)</td>
<td>70</td>
<td>Fischer (1944)</td>
</tr>
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<td>Proteinase</td>
<td><em>Bacillus licheniformis</em></td>
<td>10</td>
<td>0</td>
<td>Damodaran <em>et al.</em> (1955)</td>
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<td><em>Clostridium histolyticum</em></td>
<td>6.7</td>
<td>19</td>
<td>Kocholaty and Krejci (1948)</td>
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<td>6.7(^a)</td>
<td>30</td>
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<tr>
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<td><em>Clostridium perfringens</em></td>
<td>2</td>
<td>3</td>
<td>Maschmann (1937 b)</td>
</tr>
<tr>
<td>Organism/Issue</td>
<td>Value 1</td>
<td>Value 2</td>
<td>Reference</td>
<td></td>
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<td><em>Clostridium oedematiens</em></td>
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<tr>
<td><em>Pseudomonas aeruginosa</em></td>
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<td>0</td>
<td>Morihara (1963)</td>
<td></td>
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<tr>
<td><em>Streptococcus liquefaciens</em></td>
<td>10</td>
<td>10</td>
<td>Rabin and Zimmerman (1956)</td>
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<td><em>Aspergillus niger</em></td>
<td>10</td>
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<td>Yoshida and Nagasawa (1958)</td>
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<td>Dhar and Bose (1962)</td>
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<td><em>Penicillium cyaneo-fulvum</em></td>
<td>5</td>
<td>0</td>
<td>Singh and Martin (1960)</td>
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<td>Yeast</td>
<td>-</td>
<td>0</td>
<td>Lenney (1956)</td>
<td></td>
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<tr>
<td><em>Pisum sativum</em> (peas)</td>
<td>30ᵃ</td>
<td>7</td>
<td>Soedigdo and Gruber (1960)</td>
<td></td>
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<td>0.2</td>
<td>3</td>
<td>Brady (1961)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit kidney</td>
<td>1</td>
<td>100</td>
<td>Michlin and Rubel (1933)</td>
<td></td>
</tr>
<tr>
<td>Rat muscle</td>
<td>0.1</td>
<td>45</td>
<td>Koszalka and Miller (1958)</td>
<td></td>
</tr>
<tr>
<td>Rat muscle</td>
<td>0.1</td>
<td>5</td>
<td>Koszalka and Miller (1960)</td>
<td></td>
</tr>
<tr>
<td>Rat brain</td>
<td>10</td>
<td>97</td>
<td>Ansell and Richter (1954 b)</td>
<td></td>
</tr>
<tr>
<td>Proteinase I</td>
<td>Beef lung</td>
<td>1ᵃ</td>
<td>50</td>
<td>Dannenberg and Smith (1955)</td>
</tr>
<tr>
<td>Proteinase II</td>
<td>Beef lung</td>
<td>2ᵃ</td>
<td>34–50</td>
<td>Dannenberg and Smith (1955)</td>
</tr>
<tr>
<td>Solanain</td>
<td>Horse nettle</td>
<td>30</td>
<td>2</td>
<td>Greenberg and Winnick (1940)</td>
</tr>
<tr>
<td>Tripeptidase</td>
<td>Calf thymus</td>
<td>1</td>
<td>Stim 14ᵇ</td>
<td>Ellis and Fruton (1951)</td>
</tr>
<tr>
<td></td>
<td>Human erythrocytes</td>
<td>15</td>
<td>50</td>
<td>Tsuboi et al. (1957)</td>
</tr>
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</table>

ᵃ Iodoacetamide.
ᵇ Stim = stimulation.
bility to inhibitors varies markedly. Many of them, especially the cathepsins and those simply designated "proteinase" or "protease," are not well characterized. Despite the large number investigated, there have been very few quantitative studies of the mechanisms of inhibition. Sometimes it is difficult to determine in certain studies whether the inhibitor acts on the enzyme or on the substrate, since preincubation studies have seldom been done. Small synthetic peptides as substrates are preferable from this standpoint, but it is likely that the inhibition will often be different from that found when proteins are used. In some instances one must consider the reaction of iodoacetate with a natural activator (which may be cysteine or glutathione), as postulated by Michlin and Rubel (1933).

It was demonstrated several times in the early work that the nitroprusside reaction is reduced following reaction of iodoacetate with proteolytic enzymes. When papain is inactivated with iodoacetate, one SH group is carboxymethylated, and thus complete inhibition occurs from the addition of 1 equivalent of the inhibitor (Balls and Lineweaver, 1939 a), as is also the case with ficin (Liener, 1961). Studies with papain became confused, due mainly to the report of Ganapathy and Sastri (1939) that iodoacetate reacts readily with oxidized (disulfide) papain, leading them to postulate other groups than SH as important. They also said that the amount of iodoacetate required for inactivation is much less than to react the SH groups; however, papain apparently contains only one reactive SH group, and five additional half-cystines. More recently, iodoacetate-treated papain has been hydrolyzed and an S-carboxymethyl-L-cysteine residue detected (Finkle and Smith, 1958). E. L. Smith (1958) has suggested that the SH group in papain is perhaps a "high-energy" group, existing as a thiol ester with an adjacent carboxyl group; this may hinder reaction with certain reagents (e.g., nitroprusside and porphyrindin) but is claimed to increase reactivity to mercury and alkylating agents. When high concentrations of inhibitor are required for inactivation, one cannot be certain that only SH groups are involved, as pointed out by Maver and Thompson (1946) for lymphosarcoma cathepsin, which is inhibited 21% by 10 mM iodoacetamide.

Comparisons on a quantitative basis between iodoacetate, iodoacetamide, and o-iodosobenzoate are rare, and thus it is interesting to note the results of Tsuboi et al. (1957) on human erythrocyte tripeptidase (Fig. 1-4). Not only is iodoacetamide more potent than iodoacetate, but the inhibition-concentration curves are somewhat different in configuration. This enzyme, unfortunately, is not one of those particularly sensitive to iodoacetate; it would be interesting to know what happens to the iodoacetamide curve at lower concentrations. This enzyme is unusual also in that it is more inhibited by o-iodosobenzoate than by the alkylating agents.
Fig. 1-4. Inhibitions of human erythrocyte tripeptidase at pH 8 and 0°, incubation being carried out overnight. (From Tsuboi et al., 1957.)

**Effect of pH on Inhibitions by Iodoacetate**

We have noted (page 12) that the rate of reaction of iodoacetate with various thiols increases with increase in pH, and that the pH determines the type of group reacting in ribonuclease (page 33). With respect to enzyme inhibition, surprisingly little on pH effects has been reported. Indeed, no investigation of an iodoacetate-sensitive SH enzyme has ever been made. Maschmann (1937 a, b) studied the effects of iodoacetate at pH 5 and 7 on bacterial proteases, but these enzymes are inhibited so little that comparison at the different pH's is impossible. Schneyer (1952) found salivary amylase to be inhibited more at pH 3.5 than at 7, but this enzyme does not contain SH groups, so that these results may be a reflection of the alkylation of methionine or histidine residues. Similarly, Ramasarma et al. (1959) stated that β-glycerophosphatase is inhibited slightly at pH 3.2 but not at all at pH 5.4–9.6. Thus this early work did not support the expectation that inhibition would increase with the pH, but more recent reports correlate better with the results obtained using simple thiols. The penicillinase from *Aerobacter* is inhibited by iodoacetamide much more strongly at pH 7.4 than at 6.0 (J. T. Smith, 1963 b), the carboxydismutase catalyzing the primary CO₂ fixation in photosynthesis is inhibited by iodoacetamide more rapidly as the pH is increased from 8 to 10 (Rabin and Trown, 1964), and horse liver alcohol dehydrogenase is inhibited faster at pH 9
than at 7 (McKinley-McKee, 1963). The results with carboxydismutase suggested an enzyme group with a $pK_a$ of 8.82, which fits well with an SH group, and the authors also isolated $S$-carbamylmethylcysteine from the iodoacetamide-C$^{14}$-treated enzyme.

An interesting situation was postulated by Hagen (1957) as a result of his finding that liver cathepsin is inhibited more at lower pH's (see Table 1-8). Cysteine was added as an activator and it may be that iodoacetate reacts with it, this occurring more rapidly at higher pH's and thus depleting the iodoacetate so that less is available for inhibition of the enzyme.

**Protection of Enzymes against Iodoacetate**

Protection of 3-PGDH by substrate and various phosphorylated substances, of alcohol dehydrogenase by substrate and NAD, and of creatine kinase by either substrate with Mg$^{++}$ has been discussed. There are many other examples, some of which are given in the accompanying tabulation.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Protector</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Aldehyde dehydrogenase</td>
<td>Acetaldehyde</td>
<td>Mahler et al. (1954)</td>
</tr>
<tr>
<td>Homogentisicase</td>
<td>NAD</td>
<td>Stoppani and Milstein (1957 b)</td>
</tr>
<tr>
<td>$\beta$-Hydroxyisobutyrate</td>
<td>Homogentisate</td>
<td>Tokuyama (1959)</td>
</tr>
<tr>
<td>dehydrogenase</td>
<td>NAD</td>
<td>Robinson and Coon (1957)</td>
</tr>
<tr>
<td>Hydroxypyruvate reductase</td>
<td>Hydroxypyruvate</td>
<td>Behal and Hamilton (1962)</td>
</tr>
<tr>
<td>Prolidase</td>
<td>Mn$^{++}$</td>
<td>Davis and Smith (1957)</td>
</tr>
<tr>
<td>Pyruvate decarboxylase</td>
<td>Pyruvate</td>
<td>Stoppani et al. (1953)</td>
</tr>
<tr>
<td>Pyruvate oxidase</td>
<td>Diphosphothiamine</td>
<td>Baer (1948)</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>Cytidylate</td>
<td>Barnard and Stein (1959)</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>Fluoride + phosphate</td>
<td>Stoppani and Brignone (1956)</td>
</tr>
<tr>
<td></td>
<td>Suramin</td>
<td>Stoppani and Brignone (1957)</td>
</tr>
</tbody>
</table>

The ability of a substance to protect apparently depends primarily on its location of binding on the enzyme surface relative to the attacked SH group and the energy of binding. Thus NADP protects the NADP-dependent aldehyde dehydrogenase of yeast, but NAD does not because the latter is not bound significantly. Homogentisicase is protected by substrate but not by Fe$^{++}$, whereas $\beta$-hydroxyisobutyrate dehydrogenase is protected by NAD but not by substrate. Leucine decarboxylase is protected by pyridoxal-P but leucine actually potentiates the inhibition by iodoacetate (Sutton and King, 1959, 1962). In this case it was postulated that pyridoxal-P covers the SH group whereas binding of the substrate exposes it. Malate dehydro-
Inhibition of enzymes

Glucosgenase binds NADH less well after treatment with iodoacetamide, but NADH does not protect the enzyme (Grimm and Doherty, 1962), indicating possibly that the concentration of NADH used was not sufficient. The protection afforded by reversible inhibitors against irreversible inhibitors is illustrated by the results on succinate dehydrogenase in the tabulation above, and it may be noted that efficient protection is given only by fluoride and phosphate together, just as the inhibition by fluoride and phosphate together is greater than with either alone.

Quantitative studies on protection may have some value but most of the reports simply demonstrate protection and the authors conclude that the iodoacetate or other inhibitor must thus react with the active center, which is not such an exciting revelation. Even this conclusion is not strictly valid because protection can arise by several mechanisms (Fig. 1-5). The protector could sterically ward off iodoacetate from a vicinal SH group (I), electrostatically repel a negatively charged iodoacetate molecule (II), alter the enzyme structure to sequester an SH group (III), or actually cover the SH group at the active center (IV). In addition, a substrate might stabilize

Fig. 1-5. Possible mechanisms for the protection of enzyme active sites from iodoacetate (IA⁻) or other SH reagents.

See text for explanation.
the enzyme against secondary inactivation as it often can against enzyme denaturation. Furthermore, failure of a substance to protect does not necessarily prove that the SH group is unassociated with the binding location of the substance but might be due to (a) too low a relative affinity for the enzyme, (b) too low a concentration used, (c) inability to cover adequately the particular SH group, or (d) too long an incubation period (since a protector generally slows the rate of inhibition but does not change the final degree of inhibition).

Reversibility of Iodoacetate Inhibition

If the inhibition arises from carboxymethylation of an enzyme group, one would expect the inhibition to be very slowly reversible or completely irreversible upon removal of the iodoacetate. Most of the early workers reported irreversibility (Hopkins et al., 1938; Barron and Levine, 1952), but there have been a surprisingly large number of claims for reversibility. For example, Walsh and Walsh (1948) found fructose-1,6-diphosphatase, inhibited 40% by 10 mM iodoacetate, to be completely reactivated by cysteine, and Rocca and Ghiretti (1958) state that cysteine will completely reverse the inhibition of D-glutamate oxidase. In some instances dialysis is sufficient to reactivate, as with arylsulfatase (Maengwyn-Davies and Friedenwald, 1954) and papain (Weissmann et al., 1960). It is somewhat unexpected that Fe++ reactivates the enzyme responsible for the reaction 3-hydroxyanthranilale → quinolinate (Miyake et al., 1954).

Various possibilities for explaining such reactivation come to mind. It is quite possible that in some cases a hydrolysis of the carboxymethylcysteine bond occurs:

\[ E-S-CH_2-COON^- + H_2O \rightarrow E-SH + H^+ - CH_2COO^- \]

particularly if the pH is favorable, or possibly a thiol can react as follows:

\[ E-S-CH_2-COON^- + HS-R \rightarrow E-S-S-R + H^+ - CH_2COO^- \]

\[ E-S-S-R + HS-R \rightarrow E-SH + R-S-S-R \]

Maengwyn-Davies and Friedenwald (1954) thought that, in the case of arylsulfatase, the iodoacetate (which was 20 mM) might have reacted with the product of the reaction, p-nitrophenol, instead of on the enzyme. A more likely general explanation is that a fraction of the enzyme SH groups are in the oxidized disulfide form and cannot be carboxymethylated. Let us assume that half the SH groups are in the oxidized state and the enzyme half active; addition of a thiol activates the enzyme fully. If iodoacetate reacts completely with the free SH groups and not with the disulfide groups, the enzyme will be inhibited 100%; if a thiol is now added, the activity
will be restored. Finally, if a portion of the inhibition is due to iodine in the iodoacetate, thiol would be likely to reverse this.

Alkylation of Certain Enzymes in the Glycolytic Pathway

Iodoacetate and related alkylating agents have recently been used to characterize the active sites of enzymes. For this purpose quite high concentrations are often used so that these studies provide no information on effects obtained in cellular preparations, but the results in themselves are quite interesting and illustrate the value of these substances in the delineation of active sites. When yeast enolase is incubated with 300 mM bromoacetate for 2 hr at pH 7 and 35°, there is 90% inactivation and the carboxymethylation of 2.7 histidine residues, 3 methionine residues, and 1 lysine residue (Brake and Wold, 1962). If 2-phosphoglycerate and Mg++ are present, only 1.6 methionine residues are attacked and the enzyme activity is less reduced. Thus it appears that the inhibition of enolase by bromoacetate is due to the carboxymethylation of 1 or 2 methionine residues at the active site.

The SH groups of phosphoglucomutase treated with 67 mM iodoacetamide for 10 min at pH 8 and 25° are not carbamylmethylated, but in the presence of the substrate one SH group becomes more reactive (Koshland et al., 1962). This picture was modified somewhat by using longer incubation periods of 180 min and 270 mM iodoacetamide, from which it is seen that in the first 10 min the substrate accelerates alkylation but after that it protects, although incompletely (Koshland, 1964). It was questioned whether the protection observed is due to the presence of cysteine, methionine, and lysine at the active site, or to changes in the configuration of the enzyme brought about by glucose-6-P.

The results obtained on aldolase from rabbit muscle are puzzling. If the enzyme is first incubated with β-mercaptoethanol to ensure reduction of all available disulfide groups, the thiol then removed, and incubated with iodoacetamide in urea for 2 hr at pH 7.5 and 40°, no free SH groups are detectable (Westhead et al., 1963). If now the enzyme is retreated with β-mercaptoethanol, one SH group appears. This could be explained either by assuming that the aldolase is not homogeneous, essentially half the enzyme containing a disulfide group and half not, or by visualizing a lone SH group existing in some combined form other than disulfide.

Kinetics of Inhibition

Iodoacetate and iodoacetamide usually inactivate enzymes slowly, and sometimes very slowly, as has been known since the work of Hopkins and Morgan (1938) on succinate dehydrogenase, at which time they showed
that the discrepancy between their results and those of Dixon (1937) was due to different times of incubation. Certain enzymes, such as myosin ATPase (Bailey and Perry, 1947) and aspartase (Ellfolk, 1953), require many hours; the latter enzyme is inhibited 20% at 8 hr and 50% at 24 hr by 10–50 mM iodoacetamide. If the enzyme is not preincubated with inhibitor the results mean very little, and probably many of the low inhibitions observed are due to a failure to appreciate the time factor. Even those enzymes which react relatively rapidly with iodoacetate require some preincubation; thus, 3-PGDH from Escherichia coli is inhibited significantly more after 10 min preincubation than without preincubation (Still, 1940). It is very difficult to generalize but, for practical purposes and taking into account the usual period over which most cellular studies of glycolysis are carried out, one might say that at least a 30-min preincubation period should be used when testing an enzyme for its response to iodoacetate. Very few enzymes react completely with reasonable concentrations of iodoacetate within 30 min. The results with rice alcohol dehydrogenase (Fig. 1-6), are

Fig. 1-6. Rates of inhibition of rice alcohol dehydrogenase by iodoacetate at different concentrations. (Data from App and Meiss, 1958.)

probably quite typical, and emphasize how meaningless reported degrees of inhibition may be without accurate statements of the times of contact of the enzyme with inhibitor. Yet in over half the studies now being published, it is impossible to find mention of preincubation procedures.
Inhibition of Enzymes

Interpretation of Partial Inhibition

If the carboxymethylation of a group at the active center blocks off a substrate or coenzyme, or otherwise prevents catalysis, the enzyme would be expected to be completely inhibited if the iodoacetate were in excess and sufficient time given for reaction. In other words, under these assumptions, enzymes should be inhibited in an all-or-none fashion; i.e., either they react or they do not, and in the former case they would be completely inhibited. However, most of the results published are partial inhibitions. Partial inhibition may mean (a) insufficient time has been allowed for the reaction, (b) the iodoacetate has been used up, in reaction either with the enzyme or with other substances in the preparation, or (c) the fully reacted enzyme is not completely inhibited. Most of the results are probably explained by the insufficient time factor, especially in purified preparations in which the iodoacetate is likely to be in excess. There appear to be some cases in which a maximal inhibition is reached in the presence of adequate amounts of inhibitor, and here we must visualize a reaction of iodoacetate with a vicinal SH group, this reducing the affinity of the substrate or coenzyme for the enzyme, or slowing the breakdown of the ES complex, but not completely. It is usually not easy in a particular instance to determine the cause of partial inhibition. For example, in Fig. 1-6, one does not know if 1 mM iodoacetate will eventually inhibit completely, although it does not appear that it will. However, this was done in a homogenate containing a number of components capable of reacting with iodoacetate, so perhaps the inhibitor is depleted at this concentration. The near-complete inhibitions observed at higher concentrations could be taken as evidence that this is the case, but groups other than SH may be attacked at these high concentrations. One is also somewhat troubled by the shapes of most of the inhibition-time curves, inasmuch as usually there is an initial relatively rapid reaction, which does not inhibit the enzyme completely, followed by a slower reaction or reactions. Are these slower phases due to reaction with less reactive groups on already reacted enzyme, or to secondary inactivation unrelated to further carboxymethylation?

Summary of Enzyme Inhibition

Iodoacetate and iodoacetamide at concentrations below 5 mM and in the physiological pH range probably react with enzyme SH groups much more rapidly than with other groups, although there is always the possibility that a particular amino acid residue is in a specially reactive state. The inhibitions develop slowly and seldom reach completion before 30-60 min. In most cases the enzyme is protected by the presence of substrate or coenzyme. Inhibition is often irreversible but in some instances partial or complete reactivation may be achieved by dialysis or treatment with thios.
These facts make it evident that intracellular inhibition of an enzyme may be quite different from that of the isolated and purified enzyme.

The question of specificity of inhibition by iodoacetate must be postponed until the various metabolic pathways have been discussed, but we can see from the few enzymes mentioned that several are readily inhibited. If we arbitrarily define a sensitivity to iodoacetate as implying a significant inhibition at concentrations below 1 m\(M\), since this blocks glycolysis effectively in most cases, we note that certain alcohol dehydrogenases, succinate dehydrogenases, and proteolytic enzymes must be classed as sensitive. The results with most of the important enzymes will be given in later sections, but data on a number of enzymes of interest are summarized in Table 1-9, since these enzymes do not fall readily into any general category. It is clear that several of them are inhibited markedly at concentrations below 1 m\(M\). It is already established that a specific action on 3-PGDH or the glycolytic pathway in the broadest sense is impossible. This does not mean that under certain circumstances an inhibition of glycolysis for practical purposes is impossible, since many of the sensitive enzymes will not be involved in what is measured, but it should sound a note of caution to facile interpretation of results on cells or tissues. By no means all the enzymes tested with iodoacetate have been included in Table 1-9, or in the later tables, and we note only in passing that the following enzymes seem to be inhibited very little or not at all: acetyl phosphatase, 5’-adenylate deaminase, amylase, amylo-1,6-glucosidase, arsenite dehydrogenase, cellulase, chlorophyllase, diamin oxidase, inositol dehydrogenase, laccase, maltase, mucinase, nucleotidases, nucleotide pyrophosphorylase, oxalate decarboxylase, peroxidase, \(\alpha\)-phenylmannosidase, phosphatases, polygalacturonase, thiamine kinase, thioesterase, urease, uricase, uridine diphosphoglucose dehydrogenase, and vitamin K reductase.

**EFFECT ON ELECTRON TRANSPORT SYSTEMS**

Several NAD- and NADP-dependent dehydrogenases are inhibited by iodoacetate in reasonably low concentration. The iodoacetate is not acting on the nucleotides but on the apodehydrogenase. This was shown for the inhibition of 3-PGDH by Adler et al. (1938). Incubation of NAD with iodoacetate and starting the reaction by addition of enzyme resulted in no initial inhibition, whereas incubation of the enzyme and iodoacetate gave initial inhibition. It is likely that in all these cases the inhibition is due to carboxymethylation of SH groups on the dehydrogenases. The various components conducting electrons from NADH or NADPH to oxygen seem in general to be fairly resistant to iodoacetate. The cytochrome system is not inhibited significantly by iodoacetate up to 10 m\(M\), at least as determined with substrates (hydroquinone, ascorbate, \(p\)-phenylenediamine) feeding electrons directly into this portion of the chain (Table 1-10). The observa-
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Iodoacetate (mM)</th>
<th>% Inhibition</th>
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<td>(coenzyme-independent)</td>
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<td>Yeast (NADP-dependent)</td>
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<td>Stoppani and Milstein (1957 b)</td>
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### Table 1.9 (continued)

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<td>(1959)</td>
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<td>2&lt;sup&gt;a&lt;/sup&gt;</td>
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</tr>
<tr>
<td></td>
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<td>100</td>
<td>Revel and Racker (1960)</td>
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<td>Phosphoprotein phosphatase</td>
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<td>Kd (M)</td>
<td>Vmax (nmol/min/mg)</td>
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<td></td>
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<td>1.62</td>
<td>40</td>
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</tr>
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<td>1.762</td>
<td>20</td>
<td>Jaenicke and Brode (1961)</td>
</tr>
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</tr>
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* Iodoacetamide.
<table>
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<th>(%) Inhibition</th>
<th>Reference</th>
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<td>30(^b)</td>
<td>0</td>
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<td></td>
<td>Heart (?)</td>
<td>—(^b)</td>
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<td>Barron and Singer (1945)</td>
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<td>0-13</td>
<td>Kreke \textit{et al.} (1950)</td>
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<td>\textit{Arbacia punctulata} eggs</td>
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<td>Doi and Halvorson (1961)</td>
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<td>10</td>
<td>38</td>
<td>Vernon \textit{et al.} (1952)</td>
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<td></td>
<td>Pig liver</td>
<td>62.5</td>
<td>98</td>
<td>Garfinkel (1957)</td>
</tr>
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<td></td>
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<td>1.5(^b)</td>
<td>85</td>
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<td></td>
<td></td>
<td>125(^b)</td>
<td>100</td>
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<td>Pig liver</td>
<td>62.5</td>
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<td>Garfinkel (1957)</td>
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<td></td>
<td>1.5(^b)</td>
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<td>125(^b)</td>
<td>89</td>
<td>Ernster \textit{et al.} (1962)</td>
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<td>Mahler \textit{et al.} (1958)</td>
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<td>1(^b)</td>
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<td>b</td>
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<td>58</td>
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</tr>
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<td></td>
</tr>
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<td>9</td>
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</tr>
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<td></td>
</tr>
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<td>Spinach chloroplasts</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>NADPH:trichloroindophenol oxidoreductase</td>
<td>Spinach chloroplasts</td>
<td>3</td>
<td>60</td>
<td></td>
</tr>
</tbody>
</table>

---

*a* The terminology in this group of enzymes is confused and thus the nature of the hydrogen acceptors has been indicated in each case. Such enzymes would usually be called dehydrogenases if other substrates were involved.

*b* Iodoacetamide.
tion that iodoacetate increased some 100-fold the rate of autoxidation of cytochrome c (Boeri and Tosi, 1954) is interesting, but probably in metabolic or cellular systems this would not alter the high rate of cytochrome c oxidation via the oxidase.

Evidence that there is little effect on the components from the initial dehydrogenase to the cytochrome c is indirect and mainly on the basis that the inhibition of the entire system can be fully attributed to the action on the dehydrogenase. It is true that the oxidation of NADH and NADPH by various preparations is occasionally inhibited moderately by iodoacetate (Table 1-10), but in such systems it is difficult to know if the normal electron transport sequence involving bound endogenous nucleotides is being tested. The resistance of several metalloflavin oxidases to iodoacetate indicates little if any inhibition of these components. One can conclude from the available data that inhibitions of most oxidations are located primarily on the enzyme initially attacking the substrate when iodoacetate is 1 mM or lower, with possible minor contributions in certain cases from effects on electron transport between NADH or NADPH and cytochrome c, particularly when higher concentrations are used.

**EFFECTS ON OXIDATIVE PHOSPHORYLATION**

It was known over 30 years ago that iodoacetate inhibits the formation of ATP and creatine-P in tissues because of the depression of the oxidations supplying the energy. We shall now try to determine whether iodoacetate can affect the generation of high-energy bonds more directly, namely, through an uncoupling of oxidative phosphorylation. Most of the earlier work involved fairly complex systems and the results are not easy to interpret. Thus Kalckar (1937) found that phosphorylation is increased in cat renal cortex mince when glucose is added, and that 1.25 mM iodoacetate inhibits this completely, although depressing oxygen uptake only 37%. The phosphorylation here was not determined by ATP formation but mainly as accumulation of hexose phosphates, so that numerous sites of action might be postulated. On the other hand, cyanide inhibits phosphorylation and respiration equally. Ochoa (1941) measured phosphorylation (again as hexose phosphates) associated with pyruvate oxidation in pigeon brain dispersions and stated that 1 mM iodoacetate has no effect on the P:O ratio; however, it seems to be reduced from 2.02 to 1.73 (when fluoride is present to preserve the phosphate esters). The respiration of sea urchin egg homogenate in the presence of glucose is actually stimulated by 5 mM iodoacetate, but phosphorylation is simultaneously inhibited 14% (Lindberg and Ernster, 1948). This early work does not prove an uncoupling action but suggests it.

Oxidative phosphorylation could be investigated directly only when
mitochondrial suspensions could be used. Lehninger (1949) reported that iodoacetate does not uncouple phosphorylation associated with NADH oxidation by rat liver mitochondria, and later (Lehninger, 1951) confirmed this for \( \beta \)-hydroxybutyrate oxidation. However, he states that iodoacetamide uncouples (no data given), perhaps because it can penetrate into the mitochondria while iodoacetate cannot, which is unlikely. Kaufman (1951) also found phosphorylation to be unaffected by iodoacetate during oxidation of \( \alpha \)-ketoglutarate by a pig heart preparation. No reduction in oxidative phosphorylation by iodoacetate has been reported for beef heart mitochondria oxidizing NADPH (Joshi et al., 1957) or for sweet potato mitochondria oxidizing citrate (Hackett et al., 1960). On the other hand, there have been three reports demonstrating an uncoupling action. Phosphorylation is inhibited more than oxygen uptake in rat kidney homogenate oxidizing succinate (Hirade, 1952), and is inhibited 56\% by 1 mM iodoacetate in rat liver mitochondria oxidizing choline while oxygen uptake is unaffected (Rothschild et al., 1954). The P:O ratio of guinea pig liver mitochondria oxidizing glutamate is decreased from 2.6 to 2.1 by 0.3 mM iodoacetate (Chari-Bitron and Avi-Dor, 1959 b). Although endogenous mitochondrial phosphorylation is inhibited 70\% by 5 mM iodoacetamide (Weinbach, 1961), no data on the oxygen uptake are available.

The important problem of whether iodoacetate and iodoacetamide can uncouple oxidative phosphorylation is thus still open. Although partial uncoupling may be observed in certain instances, it seems safe to say that a true uncoupling does not occur, i.e., that phosphorylation is not so specifically blocked that oxygen uptake is released and increased, as occurs with the classic uncouplers such as 2,4-dinitrophenol.

**ANAEROBIC GLYCOLYSIS AND FERMENTATIONS**

The most important effect of iodoacetate is to depress the formation of lactate, ethanol, and other products from the breakdown of carbohydrate by an inhibition of 3-PGDH. This action can usually be measured accurately only under anaerobic conditions where the Embden-Meyerhof glycolytic pathway is solely responsible for the metabolism of glucose and related sugars. In this section we shall, therefore, consider the effects of iodoacetate on this pathway under anaerobic conditions, and in the following sections extend the treatment to aerobic situations in which other pathways may be operative. An attempt will be made to answer the following questions. (1) What is the optimal iodoacetate concentration range for the inhibition of the glycolytic pathway? (2) Is the glycolytic pathway in different organisms and tissues equally inhabitable by iodoacetate? (3) Is the site of inhibition entirely on 3-PGDH? (4) What is the chronological sequence of change brought about in cells by the action of iodoacetate? (5) Is it pos-
sible to block the glycolytic pathway selectively? It is believed that these are the basic problems in the use of iodoacetate and that only by understanding them can this inhibitor be properly applied. The answer to the last question will have to await discussion of the effects of iodoacetate on various metabolic systems (see page 167).

There has been a good deal of confusion in the terminology and methods for determination of glycolysis. In the literature one finds glycolysis measured by the formation of lactate, the formation or release of CO₂, the utilization of glucose or other carbohydrate, and occasionally the formation of various substances other than lactate. Anaerobic glycolysis is frequently equated with \( Q_{\text{CO}_2}^{\text{N}} \) on the assumption that the CO₂ driven off from a bicarbonate medium is due to the lactic acid formed. First, the increase in acidity during glycolysis is not due to the ionization of lactic acid, since at physiological pH's the lactate anion is formed from the pyruvate anion. The overall process may be represented by

\[
\text{C}_6\text{H}_{12}\text{O}_6 \rightarrow 2 \text{C}_2\text{H}_4\text{O}_4^- + 2 \text{H}^+
\]

but the hydrogen ions arise much earlier than lactate in the pathway. Second, hydrogen ions may be given off or taken up by other reactions proceeding in the preparation, e.g., in phosphate transfers. However, \( Q_{\text{CO}_2}^{\text{N}} \) is a measure of anaerobic glycolysis, whereas \( Q_{\text{CO}_2}^{\text{O}} \), which has been termed aerobic glycolysis, is by no means necessarily related to glycolysis as originally defined. It is preferable to determine lactate directly both aerobically and anaerobically. The following terminology will be used in this book. 

Glycolysis is the metabolism of sugars (particularly glucose) to lactate, and may be either aerobic or anaerobic. Glycogenolysis is the metabolism of glycogen to lactate. Fermentation is the anaerobic metabolism of any substance in microorganisms. The Embden-Meyerhof glycolytic pathway, which will be designated the EM pathway, is the sequence of reactions from glucose to pyruvate, and does not include phosphorylase, lactate dehydrogenase, or other enzymes acting on pyruvate. This may be at slight variance with previous usage, but it is felt that some term must be used to represent this pathway in discussions of the effects of iodoacetate. The pentose-P pathway is one of the routes of carbohydrate metabolism alternate to the EM pathway and involves a direct oxidation of glucose-6-P with subsequent formation of pentose-P's and triose-P.

Sensitivity of the EM Pathway to Iodoacetate

Results on the inhibition of anaerobic glycolysis and fermentations with iodoacetate and related inhibitors in a variety of organisms are given in Table 1-11. Some preparations are cellular and others acellular, different methods were used, and, perhaps more important, different incubation
times and ranges of pH were used, so that the data are not completely comparable. Nevertheless, inspection of this table shows that the EM pathway is quite sensitive to iodoacetate, and that in general it may be inhibited over 90% by concentrations of 0.1 mM or above, even in cellular preparations where penetration of the iodoacetate may be limited. Concentrations as low as 0.01 mM appear to inhibit around 40-50% and, from the work of Meyer (1932) on lactic bacteria, possibly concentrations down to around 0.0005 mM may have some effect.

What is an optimal concentration range of iodoacetate to use in blocking the EM pathway selectively? Without considering other metabolic systems, we can ask how low one can go and still expect marked block. These are dangerous questions to try to answer because the reader may accept whatever is said with too much faith. But practically one wishes to have some idea of what concentration to use in working with a new organism or tissue, and particularly to avoid higher concentrations than necessary. There are many factors which must be taken into account. (1) Are the conditions aerobic or anaerobic, since we shall see that different sensitivities may be exhibited? (2) Is the preparation cellular or acellular, i.e., are penetration and external pH factors? (3) How long an incubation period with the inhibitor can be used in the particular experiment? (4) What is the inherent sensitivity of the 3-PGDH for the preparation? (5) Are there protective substances which may either react with iodoacetate or shield the active center of 3-PGDH? (6) How much inhibition of the EM pathway is required for the particular purpose of the investigation? If one assumes an extract or homogenate where penetration is not a factor, a concentration range for iodoacetate of 0.05-0.2 mM might be suggested. In most cases this will block over 90% and higher concentrations will be unnecessary; we shall see that when the concentration approaches 1 mM or above it is difficult to achieve a selective block of the EM pathway. It is much more difficult to give an optimal range for cellular preparations, unless the cell is permeable to the iodoacetate anion or the pH is favorable for penetration of the undissociated acid. In the latter case, a range similar to that given above would be applicable, but when the intracellular concentration is less than that in the medium, the concentration to be used in the medium may have to be much greater than 0.2 mM. However, as may be seen in Table 1-11 and from the discussion of specificity in Volume I (pages 877-878), it is often possible to inhibit cellular glycolysis significantly, and reasonably specifically, at concentrations around 0.2 mM. The most important thing is not to use iodoacetate at concentrations of 1 mM or above for the inhibition of the EM pathway unless there is good evidence that this is necessary, for one reason or another. When one wishes to correlate a cell function with the operation of the EM pathway, the use of unnecessarily high concentrations of iodoacetate makes the interpretation very dif-
<table>
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<th>Organism and tissue</th>
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References:
- Link *et al.* (1952)
- Hatch and Turner (1958)
- Agosin and von Brand (1953)
- Fulton and Smith (1960)
- Cleland and Rothschild (1952 a)
- Agosin *et al.* (1957)
- Cleland (1949)
- Humphrey (1944)
- Barron and Tahmisian (1948)
- Humphrey and Siggins (1949)
- Meyerhof and Boyland (1931)
- Stannard (1938 a)
- Haarmann (1932)
- Shorr *et al.* (1938)
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<th>Organism and tissue</th>
<th>Inhibitor (^a)</th>
<th>Concentration (mM)</th>
<th>(%) Inhibition</th>
<th>Reference</th>
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<td>Krebs (1931)</td>
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<td>100</td>
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<td>67</td>
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<td>Concentration (mM)</td>
<td>% Inhibition</td>
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<tr>
<td></td>
<td></td>
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<td>100</td>
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</table>

<sup>a</sup> The inhibitors are designated as follows: IA = iodoacetate; IAM = iodoacetamide; and BrA = bro-moacetate.
difficult since specificity will, in general, be lost. Of course, it is always advisable and often necessary to obtain experimental evidence that the EM pathway under the particular conditions is being blocked to a satisfactory degree and selectivity, by methods to be discussed later.

**Comparative Susceptibility of the EM Pathway to Iodoacetate in Different Organisms and Tissues**

What has been said in the previous section refers to most mammalian and bacterial EM pathways. This pathway has been demonstrated in all types of organism, but is occasionally absent so that glucose metabolism must be through other reactions. Furthermore, several instances of resistance to inhibition by iodoacetate have been noted, as seen in Table 1-11, most of these being in invertebrates, e.g., sea urchin and oyster eggs, and cockroach and grasshopper muscles. Absence of glycolytic inhibition in fly homogenates has also been reported (Chefurka, 1954); in this case, failure to inhibit was possibly due to incubating with iodoacetate but without thiol activators, so that 3-PGDH may have been in the disulfide state and resistant. In *Streptobacterium casei*, which quantitatively converts glucose to lactate, a certain fraction of the lactate formation is resistant to iodoacetate (Field and Field, 1932). It is not known in these cases of resistance whether the particular 3-PGDH is not as readily inhibited or whether other pathways for lactate formation are present. We have noted that NADP-dependent 3-PGDH is not inhibited as easily as the NAD-dependent enzyme, and perhaps resistant enzymes occur in certain invertebrates. One must also remember that the reduction of pyruvate (formed from any source) to lactate might occur with NADH arising in any oxidation, or with NADPH by a transfer to NAD. In any event, one must be prepared to find iodoacetate-resistant EM pathways even under anaerobic conditions, and for this reason the concentrations suggested in the previous section will not always be adequate. If lactate or ethanol formation is measured, one must remember that certain organisms can anaerobically metabolize pyruvate by other pathways, e.g., rumen microorganisms LC form acetate, H₂, and CO₂ from pyruvate (Peel, 1960).

**Site of Inhibition in the EM Pathway**

The early location of the action of iodoacetate at 3-PGDH has been described in the historical summary (page 6) and we shall now inquire into the present state of this problem. In examining some of the evidence for the inhibitory site we shall encounter results which will help us understand the effects of iodoacetate in cells.

(a) *Comparison of sensitivities of EM pathway and its component enzymes.* It is difficult to compare the inhibitions of isolated 3-PGDH (Table 1-3)
with the inhibitions of the EM pathway (Table 1-11) because of the different conditions under which the experiments were run, but it is evident that the sensitivities are roughly the same. It is not necessary that they be the same, even though all the inhibition is on 3-PGDH, since in such a complex multienzyme system involving phosphorylations the inhibition on the whole system may be more than on a single component enzyme. One can only say that the EM pathway inhibitions observed can usually be attributed to an action mainly, if not entirely, on 3-PGDH. A more important question is whether other enzymes in the EM pathway are inhibited significantly at iodoacetate concentrations blocking the pathway over 90% (i.e., up to around 0.2 mM). Table 1-12 summarizes inhibitions on the enzymes of the EM pathway. There is very little evidence that any enzyme involved in the direct pathway is inhibited to an important degree at these concentrations. Even the hexokinases, probably the most sensitive enzymes other than 3-PGDH, apparently are not inhibited appreciably by 0.2 mM iodoacetate, but certainly are when the concentration is 1 mM or over. When higher concentrations of iodoacetate are used, one would expect the phosphorylation of glucose to be depressed directly (and perhaps by depletion of ATP indirectly). α-Glycerophosphate dehydrogenase is fairly sensitive also but is not on the direct pathway; however, inhibition of this enzyme may be important in the effects of iodoacetate on glycerol fermentation where dihydroxyacetone-P is reduced by NADH via this enzyme. One may conclude that iodoacetate in reasonable concentrations can exert a selective action on 3-PGDH with respect to the EM pathway. Turning to the additional enzymes involved in anaerobic glycolysis (lactate dehydrogenase, Table 1-5) and alcohol fermentation (alcohol dehydrogenase, Table 1-1), there is again little evidence that these enzymes are inhibited significantly by low iodoacetate concentrations, except possibly in some plants (e.g., Avena alcohol dehydrogenase is inhibited 73% by 0.1 mM iodoacetate) and in yeast. Although the data are not conclusive, it appears that alcohol fermentation in yeast must be inhibited by a combination of actions on 3-PGDH and alcohol dehydrogenase. Unfortunately we have no reliable data on muscle lactate dehydrogenase, so it is impossible to evaluate the importance of this enzyme in the inhibition of glycolysis in muscle.

(b) Accumulation of intermediates during iodoacetate inhibition. Inhibition of a step in a monilinear sequence of irreversible reactions leads to the accumulation of the intermediate which is the substrate for the blocked enzyme; but when the reactions are mainly reversible the situation will be more complex and the intermediates accumulating may be those some distance proximal to the block, the relative concentrations being determined in part by thermodynamic equilibria between the substances. The EM pathway is of the latter type. Accumulation of 3-phosphoglyceraldehyde would thus not be expected to be marked for two reasons. At equilibrium the ratio
### Table 1-12

**Inhibition of Enzymes in or Ancillary to the Embden-Meyerhof Glycolytic Pathway by Iodoacetate**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Iodoacetate (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
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<td>Aldolase</td>
<td><em>Trichomonas vaginalis</em></td>
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<td>0</td>
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<td>50</td>
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<td>Pea seeds</td>
<td>$1^a$</td>
<td>0</td>
<td>Stumpf (1948)</td>
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<td>0</td>
<td>Herbert <em>et al.</em> (1940)</td>
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<td>Yoshida sarcoma</td>
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<td>&lt; 3</td>
<td>Holzer <em>et al.</em> (1958)</td>
</tr>
<tr>
<td>Enolase</td>
<td>Yeast</td>
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<td>0</td>
<td>Wold and Ballou (1957)</td>
</tr>
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<td>0</td>
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<td>10</td>
<td>40</td>
<td>Walsh and Walsh (1948)</td>
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<td>69</td>
<td>Turner and Turner (1960)</td>
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<tr>
<td>Glucose-6-phosphatase</td>
<td>Pea seeds</td>
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<td>75</td>
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<td>Dixon (1937)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>60</td>
<td>Grant and Sargent (1961)</td>
</tr>
<tr>
<td></td>
<td><em>Trypanosoma rhodesiense</em></td>
<td>$1^a$</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Enzyme</td>
<td>Source</td>
<td>Iodoacetate (mM)</td>
<td>% Inhibition</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>--------------------</td>
<td>-------------------</td>
<td>--------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Rabbit muscle</td>
<td></td>
<td>0.33</td>
<td>0</td>
<td>Dixon (1937)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>42</td>
<td>van Eys et al. (1959)</td>
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<td></td>
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<td>0.1</td>
<td>23</td>
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<td>Hexokinase</td>
<td><em>Pseudomonas putrefaciens</em></td>
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<td>0</td>
<td>H.P. Klein (1953)</td>
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<tr>
<td></td>
<td></td>
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<td>26</td>
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<tr>
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<tr>
<td></td>
<td><em>Bacillus</em> sp. (strain W-2)</td>
<td>2</td>
<td>71</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>94</td>
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<tr>
<td></td>
<td><em>Neurospora crassa</em></td>
<td>10</td>
<td>47</td>
<td>Medina and Nicholas (1957 a)</td>
</tr>
<tr>
<td></td>
<td>Yeast</td>
<td>5</td>
<td>20</td>
<td>Bailey and Webb (1948)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wheat germ</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>Saltman (1953)</td>
</tr>
<tr>
<td>Phosphoenolpyruvate carboxylase</td>
<td>Yeast</td>
<td>10</td>
<td>6</td>
<td>Cannata and Stoppani (1963 c)</td>
</tr>
<tr>
<td>Phosphoenolpyruvate carboxytransphosphorylase</td>
<td><em>Propionibacterium shermanii</em></td>
<td>4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>Siu and Wood (1962)</td>
</tr>
<tr>
<td>Phosphoglucoisomerase</td>
<td>Yeast</td>
<td>10</td>
<td>0</td>
<td>Noltmann and Bruns (1959)</td>
</tr>
<tr>
<td></td>
<td><em>Aspergillus niger</em></td>
<td>10</td>
<td>0</td>
<td>Singh (1959)</td>
</tr>
<tr>
<td></td>
<td><em>Trichinella spiralis</em></td>
<td>2</td>
<td>0</td>
<td>Mancilla and Agosin (1960)</td>
</tr>
<tr>
<td></td>
<td><em>Phaseolus radiatus</em> (green gram)</td>
<td>2</td>
<td>0</td>
<td>Ramasarma and Giri (1956)</td>
</tr>
<tr>
<td>Enzyme</td>
<td>Source</td>
<td>pH 8–9</td>
<td>pH 5.7</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-------------------------------</td>
<td>--------</td>
<td>--------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Phosphoglucomutase</td>
<td>Molgula (tunicate)</td>
<td>4</td>
<td>0</td>
<td>Sable and Calkins (1953)</td>
</tr>
<tr>
<td></td>
<td>Human erythrocyte</td>
<td>20a</td>
<td>0</td>
<td>Tsuboi et al. (1958)</td>
</tr>
<tr>
<td></td>
<td>Rabbit muscle at:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH 8–9</td>
<td>2</td>
<td>33</td>
<td>Milstein (1961)</td>
</tr>
<tr>
<td></td>
<td>pH 5.7</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Phosphoglyceryl kinase</td>
<td>Pea seeds</td>
<td>6.9</td>
<td>0</td>
<td>Axelrod and Bandurski (1953)</td>
</tr>
<tr>
<td></td>
<td>Rabbit muscle</td>
<td>4.36a</td>
<td>0</td>
<td>Rao and Oesper (1961)</td>
</tr>
<tr>
<td>Phosphoglyceryl mutase</td>
<td>Rabbit muscle</td>
<td>0.7</td>
<td>0</td>
<td>Oesper (1955)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>60–70</td>
<td>Cowgill and Pizer (1956)</td>
</tr>
<tr>
<td>Phosphohexokinase</td>
<td>Pea seeds</td>
<td>5</td>
<td>51</td>
<td>Axelrod et al. (1952)</td>
</tr>
<tr>
<td>Phosphomannoisomerase</td>
<td>Pig erythrocytes</td>
<td>—</td>
<td>0</td>
<td>Bruns and Noltmann (1958)</td>
</tr>
<tr>
<td>Phosphorylase</td>
<td>Lobster muscle</td>
<td>—</td>
<td>0</td>
<td>Cowgill (1959)</td>
</tr>
<tr>
<td></td>
<td>Rabbit muscle</td>
<td>10</td>
<td>0</td>
<td>Cori et al. (1939)</td>
</tr>
<tr>
<td>Pyruvate carboxylase</td>
<td>Chicken liver</td>
<td>0.5a</td>
<td>4</td>
<td>Keech and Utter (1963)</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>Yeast</td>
<td>10</td>
<td>72</td>
<td>Washio and Mano (1960)</td>
</tr>
<tr>
<td></td>
<td>Human erythrocytes</td>
<td>1</td>
<td>28</td>
<td>Solvonuk and Collier (1955)</td>
</tr>
<tr>
<td>Uridine disphosphogalactose</td>
<td>Calf liver</td>
<td>0.1</td>
<td>6</td>
<td>Isselbacher (1958)</td>
</tr>
<tr>
<td>pyrophosphorylase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uridine disphosphoglucose</td>
<td>Pea seeds</td>
<td>10</td>
<td>0</td>
<td>Turner and Turner (1958)</td>
</tr>
<tr>
<td>pyrophosphorylase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a Iodoacetamide.
of the triose-P's is given by (dihydroxyacetone-P)/(3-phosphoglyceraldehyde) = 22, so that most of the triose-P that did accumulate would be in the form of dihydroxyacetone-P. Also the aldolase reaction is reversible with the equilibrium favoring fructose-1,6-diP, according to (fructose-1,6-diP)/(3-phosphoglyceraldehyde) \( \approx 700 \). Although the phosphofructokinase reaction is not reversible, fructose-6-P will be reformed, if there is fructose-1,6-diphosphatase activity, and hence glucose-6-P, since (glucose-6-P)/(fructose-6-P) = 2.3. One would predict a block of 3-PGDH to lead to the accumulation to varying degrees of several phosphorylated compounds, fructose-1,6-diP probably being dominant in most instances.

Inhibition of 3-PGDH should bring about changes in the NAD : NADH ratio. The addition of glucose-6-P or fructose-1,6-diP to extracts of *Saccharomyces carlsbergensis* causes a sinusoidal variation in NADH level for several minutes until a steady state is reached (Chance et al., 1964). These oscillations are prevented by 3 mM iodoacetate, and this is attributed to an inhibition of 3-PGDH with consequent oxidation of NADH to NAD by the accumulated acetaldehyde. This also provides evidence that the fluctuations in the NAD : NADH ratio are mediated through 3-PGDH.

Another factor to be considered in accumulation of hexose phosphates is the supply of ATP and other high-energy phosphate. If 3-PGDH is completely inhibited, phosphorylation will proceed until ATP is depleted to a low level, so that the total amount of hexose phosphates accumulated will depend on the phosphorylation potential of the particular tissue or preparation. When glycogen can serve as a substrate, hexose monophosphates can form readily if the supply of inorganic phosphate is adequate and phosphorlase is active. Possibly the patterns of accumulation during iodoacetate block will be somewhat different when glucose or glycogen is the initial substrate for the EM pathway.

In his initial work with iodoacetate, Lundsgaard (1930 a) observed the increase in "lactacidogen," which he interpreted as hexose phosphates, in iodoacetate-poisoned muscle, this increase corresponding to a decrease in "phosphagen," which we now know to be ATP and creatine-P. Lohmann (1931) soon showed that hexose mono- and diphosphates do indeed accumulate in the muscles of frogs given iodoacetate, and Meyerhof and Kiesling (1933) succeeded in isolating a small amount of triose-P from muscle extracts incubated with iodoacetate. The situation in glycogenolysis is well seen in the work of Ostern et al. (1939), who found glycogen to increase the esterification of inorganic phosphate in the presence of iodoacetate. The first quantitative analyses of accumulation were reported by Geiger (1940) in rat brain extracts (see accompanying tabulation). Fructose-1,6-diP increased almost 10-fold and 3-phosphoglyceraldehyde 4-fold during 30-min incubation with 0.2 mM iodoacetate. The gain in inorganic phosphate indicates the activity of phosphatases. It is evident that the disappearance of
ATP (− 6.54) is approximately balanced by the increased phosphates (+ 6.76).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration of phosphates</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Inorganic phosphate</td>
<td>11.32</td>
<td>14.36</td>
</tr>
<tr>
<td>Fructose-1,6-diP</td>
<td>0.29</td>
<td>2.81</td>
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<tr>
<td>Difficultly hydrolyzable</td>
<td>0.31</td>
<td>0.95</td>
</tr>
<tr>
<td>phosphates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-Phosphoglyceraldehyde</td>
<td>0.18</td>
<td>0.74</td>
</tr>
<tr>
<td>ATP</td>
<td>7.78</td>
<td>1.24</td>
</tr>
</tbody>
</table>

Several other more recent quantitative studies will be briefly presented in order to illustrate the variations between different organisms and tissues. During fermentation of glucose by *Streptomyces olivaceus* the concentrations of the intermediates change about as expected when iodoacetate is present (see accompanying tabulation) (Maitra and Roy, 1959). However, the relative accumulations certainly do not conform to thermodynamic predictions and the deviations may have resulted from phosphatase activity, since the experiments were run over 96 hr. Even 7.5 mM iodoacetate appears to block 3-PGDH fairly selectively over 20 min from the pattern of change in the intermediates under aerobic conditions in yeast, where a greater ATP supply probably facilitates phosphorylation of glucose (Holzer *et al.*, 1955 a).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration of phosphates</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Iodoacetate (1 mM)</td>
</tr>
<tr>
<td>Glucose-1-P</td>
<td>1.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Fructose-6-P</td>
<td>2.2</td>
<td>4.2</td>
</tr>
<tr>
<td>Fructose-1,6-diP</td>
<td>6.2</td>
<td>12.1</td>
</tr>
<tr>
<td>3-Phosphoglyceraldehyde</td>
<td>4.5</td>
<td>10.5</td>
</tr>
<tr>
<td>Phosphoenolpyruvate</td>
<td>1.3</td>
<td>1.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration of intermediates</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Iodoacetate (7.5 mM)</td>
</tr>
<tr>
<td>Fructose-1,6-diP</td>
<td>393</td>
<td>2690</td>
</tr>
<tr>
<td>Dihydroxyacetone-P</td>
<td>330</td>
<td>630</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>1.3</td>
<td>0.08</td>
</tr>
<tr>
<td>α-Ketoglutarate</td>
<td>3.3</td>
<td>0.6</td>
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The very marked accumulation of fructose-1,6-diP (see accompanying tabulation) indicates the ability of the EM pathway to proceed through the earlier stages at this high concentration of iodoacetate. A similar picture has been found in *Chlorella* exposed to 0.1 mM iodoacetate aerobically in the dark (see tabulation below) (Kandler *et al.*, 1961). Accumulation of triose-P’s also occurs, as shown in Fig. 1-7 where the rapid changes in various intermediates and ATP may be observed. It was concluded that 0.1 mM iodoacetate produces here a fairly specific block at the 3-PGDH level. The formation of $^{32}$P-labeled compounds in *Helodea densa* during 2-min incubation in the dark following 9-min exposure to 0.4 mM iodoacetate was

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration of phosphates</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Iodoacetate (0.1 mM)</td>
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<tr>
<td>Hexose monophosphates</td>
<td>65</td>
<td>45</td>
</tr>
<tr>
<td>Fructose-1,6-diP</td>
<td>6.5</td>
<td>41</td>
</tr>
<tr>
<td>Uridinediphosphogluucose</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>3-Phosphoglycerate</td>
<td>9</td>
<td>2.5</td>
</tr>
<tr>
<td>Phosphoenolpyruvate</td>
<td>5</td>
<td>0.5</td>
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</tbody>
</table>

Fig. 1-7. Effects of 0.3 mM iodoacetate on various intermediates and nucleotides in *Chlorella pyrenoidosa*, after 1 hr incubation at pH 5.5 in a medium containing glucose. (From Kandler *et al.*, 1961.)
determined by Simonis and Weichart (1958), and the results again point to a block of 3-PGDH (see accompanying tabulation). The relative accumulations and depletions here are more in accord with equilibrium conditions than in most studies, perhaps because the experimental period is shorter. Glucose is metabolized anaerobically as fast as it enters the yeast cell, since no free intracellular glucose can be detected, but 1 mM iodoacetate was claimed by Cirillo (1962) to lead to glucose accumulation up to 40-55 mM in the cell water, which implies a lack of ATP or a particularly high hexosephosphatase activity.

Ehrlich ascites carcinoma cells are very sensitive to iodoacetate and at 0.025 mM the glycolysis is inhibited around 75% under anaerobic conditions (Holzer et al., 1955 b). The accumulation of fructose-1,6-diP is very marked (see accompanying tabulation). Kvamme (1958 a, b) confirmed the accumulation of fructose-1,6-diP in these cells treated with 0.05 mM iodoacetate, but the magnitude of the effect was generally less. One interesting fact is illustrated in this work: the higher the glucose concentration, the less the effect of iodoacetate on the accumulation of fructose-1,6-diP, since the initial level rises with glucose concentration. Fructose-1,6-diP under other conditions may fail to accumulate in ascites cells, as in the experiments of Wu and Racker (1959) where no rise of this substance was observed, although lactate formation was almost completely blocked. However, the ATP level anaerobically in the presence of 0.1 mM iodoacetate was zero.
and essentially no glucose was taken up, so little of the phosphate ester could be formed, although a greater fraction of the glucose metabolized went to fructose-1,6-diP in the inhibited cells. Some fructose-1,6-diP accumulated aerobically.

The changes in erythrocytes are basically the same but differ in details from the cells previously discussed, especially with respect to 2,3-diphosphoglycerate which is normally present in relatively high concentrations. Iodoacetate causes a fall in the level of 2,3-diphosphoglycerate and a simultaneous rise in inorganic phosphate in human erythrocytes (Pranker and Altman, 1954), but since 30 mM was used it is difficult to attribute the results to a selective action of 3-PGDH. More striking results (see accompanying tabulation) with a much lower iodoacetate concentration were obtained by Mills and Jones (1961), who suggested that although iodoacetate would prevent ATP synthesis from glucose, the 2,3-diphosphoglycerate might be hydrolyzed to 3-phosphoglycerate which could form ATP during conversion to pyruvate. The 2,3-diphosphoglycerate certainly disappears but the ATP drops to very low levels, so that the authors concluded that this mechanism for ATP generation is not very important for some reason. The decrease in fructose-1,6-diP is surprising and may be due to the long incubation period (5 hr), the low levels of ATP for glucose phosphorylation, and phosphatase activity. Somewhat different results were reported by Gerlach and Lübben (1959) using shorter incubations (45 min), and here fructose-1,6-diP and triose-P accumulate typically, while there is little change in 2,3-diphosphoglycerate (Fig. 1-8). Glucose is normally metabolized in erythrocytes about as rapidly as it enters the cells and the steady-state glucose concentration is low (unless external glucose concentration is high), but in the presence of 1 mM iodoacetate the intracellular glucose rises markedly — e.g., from 16 to 393 mg% in around 5 hr — in rabbit erythrocytes (Laris, 1958), showing that phosphorylation of glucose is interfered with, although whether this is a direct action or due to ATP depletion is not known. An increase in intracellular glucose to the same concentration as in the medium has also been observed in isolated lymph node cells incubated with 1 mM iodoacetate (Helmreich and Eisen, 1958). Avian erythrocytes are much less

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration of phosphates</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Iodoacetate (0.5 mM)</td>
</tr>
<tr>
<td>Fructose-1,6-diP</td>
<td>33.5</td>
<td>24.5</td>
</tr>
<tr>
<td>2,3-Diphosphoglycerate</td>
<td>187</td>
<td>57</td>
</tr>
<tr>
<td>Monophosphoglycerates</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Inorganic phosphate</td>
<td>72</td>
<td>472</td>
</tr>
</tbody>
</table>
sensitive to iodoacetate (Gerlach and Lübben, 1959), but some accumulation of triose-P and hexose phosphates occurs with 0.25 mM bromoacetate (Dische and Ashwell, 1955).

The results on the accumulation of intermediates do not establish conclusively that the sole site of iodoacetate action is 3-PGDH, but in general conform to the pattern expected if 3-PGDH is the most susceptible enzyme. Looked at in another way, the results provide no sound evidence that iodoacetate acts on any enzyme other than 3-PGDH in the EM pathway at concentrations below 1 mM.

(c) Variation of the inhibition with the substrate used. If iodoacetate acts to block 3-PGDH only, one might expect that the formation of lactate (or ethanol) from each sugar and each intermediate before 3-phosphoglyceraldehyde in the EM pathway would be inhibited to the same degree. However, a more thorough analysis of the situation shows that this is not necessarily true. Most of the work on the relative inhibitions with different substrates has been done under aerobic conditions, and here the problem of alternate pathways complicates the results (see page 101). Nevertheless, since the aerobic experiments have been used to provide evidence for the site of iodoacetate action, they must be considered briefly along with the small amount of work done anaerobically.

Prasad (1935 a) observed that iodoacetate has less effect on endogenous glycogenolysis than on the glycolysis of exogenous glucose in cat intestine, and attributed this difference to the slow penetration of iodoacetate into the muscle cells. Kerly and Bourne (1940) also found glycogenolysis to be

---

**Fig. 1-8. Effects of 0.5 mM iodoacetate on the levels of various substances in human erythrocytes at 37°. (From Gerlach and Lübben, 1959.)**
inhibited less than glycolysis by 0.05 mM iodoacetate in retinal extracts (57% and 91%, respectively). More recently Brady et al. (1961) have reported that, whereas iodoacetate at concentrations around 0.1 mM inhibits anaerobic glycolysis from added glucose in yeast, the endogenous glycogenolysis activated by azide is either unaffected or actually stimulated. They felt that this lends support to the idea that iodoacetate depresses yeast fermentation by an action at some step between glucose and fructose-1,6-diP, rather than entirely on 3-PGDH, perhaps by preventing glucose assimilation or inhibiting hexokinase.

Although Yamasaki (1930) found the fermentation of fructose-1,6-diP by yeast to be inhibited strongly by 0.9 mM iodoacetate (probably as much as the fermentation of glucose), others have found the fermentation of fructose-1,6-diP to be less sensitive. Beevers (1950) reported that 0.1 mM iodoacetate inhibits aerobic glucose fermentation 82.5% and fructose-1,6-diP fermentation 15% at 40 min. and presented curves showing that glucose fermentation is well inhibited between 0.01 and 0.1 mM whereas fructose-1,6-diP fermentation is essentially unaffected. He postulated that some enzyme between glucose and fructose-1,6-diP must be sensitive to iodoacetate. On the other hand, Kelly et al. (1955) found the oxidation of fructose-1,6-diP by adrenals to be inhibited more than the oxidation of hexoses or hexose phosphates (see accompanying tabulation). This is to be expected if an active pentose-P pathway exists, and this was confirmed by showing that pyruvate is formed from fructose-1,6-diP but not from glucose or the hexose monophosphates in the presence of iodoacetate. It may also be mentioned that Holzer et al. (1955 b) found anaerobic glycolysis to be inhibited to the same degree by iodoacetate when either glucose or fructose is the substrate for ascites cells.

These apparently discrepant results may be explained in a variety of ways. Beevers (1950) determined the CO₂ formation by yeast rather than the appearance of ethanol, and it was previously pointed out that this is not necessarily a reliable measure of fermentation or the EM pathway. Possibly some of the CO₂ formed after addition of fructose-1,6-diP arose from hydrolysis of the compound and the consequent acidification, this

<table>
<thead>
<tr>
<th>Substrate</th>
<th>% Inhibition by iodoacetate 0.54 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>50</td>
</tr>
<tr>
<td>Fructose</td>
<td>50</td>
</tr>
<tr>
<td>Glucose-1-P</td>
<td>26</td>
</tr>
<tr>
<td>Fructose-6-P</td>
<td>8</td>
</tr>
<tr>
<td>Fructose-1,6-diP</td>
<td>93</td>
</tr>
</tbody>
</table>

active pentose-P pathway exists, and this was confirmed by showing that pyruvate is formed from fructose-1,6-diP but not from glucose or the hexose monophosphates in the presence of iodoacetate. It may also be mentioned that Holzer et al. (1955 b) found anaerobic glycolysis to be inhibited to the same degree by iodoacetate when either glucose or fructose is the substrate for ascites cells.

These apparently discrepant results may be explained in a variety of ways. Beevers (1950) determined the CO₂ formation by yeast rather than the appearance of ethanol, and it was previously pointed out that this is not necessarily a reliable measure of fermentation or the EM pathway. Possibly some of the CO₂ formed after addition of fructose-1,6-diP arose from hydrolysis of the compound and the consequent acidification, this
not being inhibitable by iodoacetate. We must also consider the over-all reactions when different substrates are used:

\[
\text{Glucose} + 2 \text{ADP} + 2 \text{P}_i \rightarrow 2 \text{pyruvate} + 2 \text{ATP} \\
\text{Glycogen} + 3 \text{ADP} + 3 \text{P}_i \rightarrow 2 \text{pyruvate} + 3 \text{ATP} \\
\text{Fructose-1,6-diP} + 4 \text{ADP} + 4 \text{P}_i \rightarrow 2 \text{pyruvate} + 4 \text{ATP}
\]

Since the ATP, ADP, and P\(_i\) levels control glycolysis and determine which reactions will be limiting, the effects of iodoacetate may be different with these three substrates. For example, if the ATP level is low, inhibition with glucose as the substrate might be greater than with fructose-1,6-diP because the former produces only half as much ATP as the latter. In other words, iodoacetate would deplete the ATP faster with glucose as the substrate and hence bring about a secondary inhibition of the hexokinase reaction. Racker (1954 a, b) has pointed out that in multienzyme systems the results of inhibitions may be more complex than is usually supposed, and that in the present case a partial inhibition of 3-PGDH may have a more marked effect on glucose metabolism than on fructose-1,6-diP metabolism. In a system where no ATP has been added and where ATP may be hydrolyzing, the inhibition of ATP generation (although by no means necessarily complete) may cause an almost total inhibition of glucose phosphorylation and hence of glucose breakdown, whereas the inhibition of the metabolism of fructose-1,6-diP will be essentially the same as is exerted on 3-PGDH. Perhaps if Beevers had added ATP to his extracts he would have observed different results. The addition of ATP to cockroach muscle fibers glycolyzing anaerobically relieves completely the inhibition by iodoacetate (Barron and Tahmisian, 1948).

Another factor to be taken into account is the rapidity with which 3-phosphoglyceraldehyde is formed and the level of concentration it reaches during the inhibition, inasmuch as 3-phosphoglyceraldehyde protects the enzyme 3-PGDH, the more effectively the higher the concentration. In most cases there was no preincubation with iodoacetate, so the inhibitor must act on 3-PGDH in the presence of the particular level of 3-phosphoglyceraldehyde occurring with the substrate used. It is possible that under certain conditions fructose-1,6-diP may form 3-phosphoglyceraldehyde more rapidly than glucose, and the 3-PGDH will be to some extent protected against iodoacetate. That the steady-state level of 3-phosphoglyceraldehyde may determine the degree of inhibition by iodoacetate is suggested by the results of Holzer and Holzer (1953). The inhibition of anaerobic fermentation of glucose by yeast was determined after incubation of the yeast either in air or in nitrogen. The inhibition was greater following anaerobic incubation, which was attributed to the lower concentration of 3-phosphoglyceraldehyde under anaerobic conditions. This mechanism may also explain the
early observation of Ehrenfest (1932) that 0.1 m\(M\) iodoacetate inhibits yeast fermentation completely at pH 4.6 if added some time before glucose, whereas when both iodoacetate and glucose are added together the inhibition develops very slowly.

**Patterns of Anaerobic Glucose Utilization in the Presence of Iodoacetate**

Even though the EM pathway is blocked at 3-PGDH, glucose may still be metabolized to a certain extent if ATP is available. We have seen that hexose phosphates may be formed and accumulate during iodoacetate block. In the anaerobic experiments of Stickland (1956 b) with yeast, of a total of 1.50 mg/vessel of glucose utilized, 1.07 was fermented, 0.24 went to polysaccharide, and 0.19 was unaccounted for. In the presence of 0.25 m\(M\) iodoacetate, 0.21 mg/vessel of glucose was utilized, 0.07 being fermented and none going to polysaccharide, leaving 0.14 unaccounted for. There is thus a strong inhibition of polysaccharide synthesis in yeast, as well as a 93% block of fermentation. The unaccounted for fraction may be hexose phosphates, but it appears likely that ATP is reduced to levels not supporting active phosphorylation, unless there is some inhibition of the pathway for polysaccharide synthesis.

A greater discrepancy between inhibition of anaerobic glycolysis and glucose uptake is seen in duck erythrocytes (Tosteson and Johnson, 1957). Iodoacetate at 1 m\(M\) reduces lactate formation from 2.6 to 0.1 millimole/liter of RBC/hr, but depresses glucose uptake only from 2 to 1 millimole/liter of RBC/hr, indicating that it is being metabolized. Since it is unlikely that glycogen formation can account for the glucose taken up, one is left with the alternative generation of hexose phosphates. One wonders also how much glucose taken up could be accounted for by a rise in intracellular glucose concentration, as has been shown to occur in other cells (see page 136). In lymphatic cells and lymphosarcoma, the inhibitions of glucose uptake and lactate formation anaerobically are not greatly different; if anything, glucose utilization is inhibited slightly more (Villavicencio and Barron, 1957).

Summarizing this meager evidence, it appears that glucose has a very limited metabolism when 3-PGDH is blocked, and that phosphorylation to an extent determined by ATP supply is the primary pathway. However, one must remember that Stickland (1956 b) found 67% of the glucose utilized unaccounted for, and we are ignorant of the products of this metabolism.

**Changes in \(pH\) during Iodoacetate Inhibition**

Anaerobic glycolysis usually leads to a decrease in the \(pH\) due to metabolically produced protons:

\[
\text{Glucose} + 2 \text{ADP} + 2 \text{P}_i \rightarrow 2 \text{lactate}^- + 2 \text{ATP} + 2 \text{H}^+ 
\]
The formation of ATP will also tend to decrease the pH since ATP \((\text{pK}_{a2} = 6.48)\) is a somewhat stronger acid than \(\text{P}_i\) \((\text{pK}_{a2} = 7.2)\) and not much weaker than ADP \((\text{pK}_{a2} = 6.26)\) (the \(\text{pK}_{a2}\)'s for ADP and ATP refer to the terminal secondary ionization), but if ATP is being utilized or hydrolyzed it will not contribute to the pH changes. In alcoholic fermentation there is no metabolic proton release, but the pH may decrease slightly as a result of the formation of ATP and CO\(_2\). When glucose is completely oxidized through the tricarboxylate cycle, the only pH changes will be due to phosphorylations or CO\(_2\). Iodoacetate by blocking 3-PGDH should prevent the fall in pH occurring in anaerobic glycolysis and may actually cause an increase in the pH resulting from the hydrolysis of ATP and creatine-P, although the formation of hexose phosphates may to some extent counteract this (the hexose phosphates are stronger acids than \(\text{P}_i\)).

The effect of iodoacetate on muscle pH was first observed by Lundsgaard (1930 a). He had observed the acidification of muscles forming lactate but in iodoacetate the acid shift was abolished and in some cases the pH increased slightly, even though the muscles were in rigor. Meyer (1932) similarly found that iodoacetate prevents the fall in pH associated with glycolysis in lactic bacteria (see accompanying tabulation, where the initial

<table>
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<th>Iodoacetate (mM)</th>
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<tbody>
<tr>
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<td>4.4</td>
</tr>
<tr>
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</tr>
<tr>
<td>0.1</td>
<td>6.8</td>
</tr>
<tr>
<td>0.2</td>
<td>7.1</td>
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</table>

pH was 7.1 and incubation proceeded for 3 hr). If rats are killed by tracheal occlusion, cyanide, or carbon monoxide the pH of their muscles falls progressively over several hours, but the muscles of animals poisoned with iodoacetate show an initial rise in pH followed by a slow return toward the normal value (Voegtlín et al., 1934). Tetanic stimulation of frog sartorius treated with iodoacetate leads to a rise in pH of 0.26 unit compared to untreated muscle (Dubuisson and Schulz, 1938), and stimulation of muscle exposed to 1 mM iodoacetate for 83 min results in elevation of the pH, attributed to the splitting of creatine-P (Jöbbsis, 1963). More marked increases in pH have been observed in suspensions of human erythrocytes (Flynn and Maizels, 1949), \(E. \text{coli}\) (Roberts et al., 1949), and Ehrlich ascites carcinoma cells (Racker, 1956; Kvamme, 1958 b) incubated with iodoace-
tate, in most cases the rise in pH being around 0.5–1 unit. There is thus adequate evidence from a variety of organisms and tissues that the predicted changes in pH actually occur. However, since there are several factors which determine these pH changes and the interrelationships are rather complex, it should not be concluded that such changes as have been observed will invariably occur.

The importance of the effects of iodoacetate on pH cannot be overlooked. The penetration of iodoacetate into cells depends on the external and internal pH's so that modification of these can secondarily affect the inhibition produced by iodoacetate, as pointed out by Beevers and Simon (1949). Furthermore, cell function can be altered through the pH changes induced by iodoacetate. Sandow and Karczmar (1950) have discussed the latency phenomena of muscle as related to normal pH changes on stimulation and the changes brought about by iodoacetate. It is probably worthwhile to remember that the internal pH of cells treated with iodoacetate may rise, and that this can be a factor in the metabolic or functional changes observed.

Chronological Sequence of Events brought About Anaerobically by Iodoacetate

The effects of iodoacetate on the EM pathway will be summarized by discussing the sequence of changes which would be predicted if iodoacetate blocked completely and specifically 3-PGDH under anaerobic conditions (the aerobic situation will be treated on page 139). The simplified scheme of anaerobic glycolysis (Scheme 1) will help to visualize these effects. The phosphatase reactions have also been included since they may be important in relation to accumulation of hexose phosphates. The over-all reaction is the generation of 2 moles of ATP from each mole of glucose utilized. This scheme also indicates the major controls of the glycolytic rate. One should perhaps also include the following reactions:

\[ \text{ATP} \rightarrow \text{ADP} + P_i \ (\text{ATPase or functional utilization}) \]

\[ \text{ATP} + \text{creatine} \leftrightarrow \text{ADP} + \text{creatine-P} \]

As the \((\text{ATP})/(\text{ADP})\) ratio increases from 0 to 1, the glycolytic rate will increase and then decrease, there being an optimal ratio for the operation of the EM pathway. The \((\text{NAD})/(\text{NADH})\) ratio is similarly important.

It is impossible to describe the changes consequent to complete block of 3-PGDH in entirely sequential fashion, inasmuch as many things occur simultaneously but, broadly speaking, there is a changing pattern.

(A) There is a simultaneous cessation of (1) oxidation of 3-phosphoglyceraldehyde, (2) formation of 1,3-diphosphoglycerate, (3) generation of ATP, and (4) reduction of NAD.
(B) The steady-state levels of 3-phosphoglyceraldehyde and dihydroxyacetone-P will remain the same or increase somewhat, while the hexose phosphates will continue to be formed as long as there is ATP and will increase in concentration roughly according to equilibria between them.

(C) The ATP level will fall due to hexose phosphorylation (and perhaps to ATPase activity or utilization of the ATP in other ways), and this will
bring about a loss of creatine-P to help maintain the ATP level. When the ATP concentration reaches a low level, hexose phosphorylation will slow and eventually cease, resulting in some cases in a rise in intracellular glucose.

(D) The fall in ATP may also reduce the total NAD and NADP concentrations by shifting the equilibria in favor of NMN, although Holzer et al. (1958) observed no fall in NAD in ascites cells inhibited by iodoacetate, nor did Hofmann (1960) in glycolyzing and nonglycolyzing yeast treated with 20 mM iodoacetate, although the (NAD)/(NADH) ratio increased markedly.

(E) Phosphatase activity will reduce the levels of hexose phosphates and release $P_i$, this acting overall to increase $P_i$ concentration.

(F) The steady-state levels of the intermediates distal to 3-PGDH will decrease and probably drop to zero, except for pyruvate, which may remain because of a deficiency of NADH to reduce it to lactate.

(G) The pH will tend to rise due to the inhibition of metabolic proton release and the breakdown of ATP and creatine-P.

(H) It is possible, although never demonstrated, that, in some cells with glucose-6-P dehydrogenase activity, gluconate-P and NADPH will be formed to a limited extent. If there are substances present to oxidize the NADPH, this reaction may proceed.

(I) All reactions outside of the EM pathway which require ATP (syntheses and cell functions) will fail due to the depletion of ATP. Such effects can bring about a variety of secondary alterations, e.g., leakage of ions from the cells, which can further modify metabolism.

We have assumed complete inhibition of 3-PGDH. The question arises as to how much 3-PGDH must be depressed before glycolysis fails, i.e., before ATP breakdown exceeds its generation. If 3-PHDG is inhibited 50%, the balance of ATP used and formed is zero, so that presumably glycolysis could continue (with lactate formation 50% depressed) if the ATP were used 100% efficiently. Since the efficiency is probably never 100% and since there are ATPase and phosphatases, glycolysis will proceed to decrease from 50% to eventually 0%. Thus 3-PGDH cannot be inhibited very much before ATP begins to fall and glucose utilization is suppressed. Much of the progressive inhibition often observed in glycolytic and functional measurements, which is usually attributed solely to the slow rate of reaction of iodoacetate, is probably due to this factor of ATP balance. It also explains why a tissue will fail faster when it is active in any way, i.e., when it is utilizing ATP.
TRICARBOXYLATE CYCLE

The effects of iodoacetate on pyruvate oxidation and the tricarboxylate cycle will be considered preparatory to a discussion of the effects on respiration and aerobic carbohydrate metabolism. The end product of the EM pathway as here defined is pyruvate, and the most important aspect of exergonic carbohydrate oxidation is the disposal pathways for this pyruvate, the commonest being the cycle (as previously, "cycle" will refer only to the Krebs tricarboxylate cycle). The central role of the cycle in energy production and the multiplicity of the types of enzyme it contains always make the cycle a fascinating subject for the study of inhibitors. Several cycle enzymes are usually classed as SH enzymes and two cofactors, coenzyme A and lipoate, also contain SH groups, so that the cycle would not be expected to be immune to iodoacetate.

Antagonism of Iodoacetate Inhibition by Pyruvate, Lactate, and Acetate

The early workers were more concerned with the ability of lactate and related substrates to counteract metabolic depression by iodoacetate than with the direct effects of iodoacetate on the oxidation of these substrates. Fundamentally, the increase in respiration previously inhibited by iodoacetate (in the presence of glucose or endogenous substrates) brought about by the addition of C2-C4 acids is not a true antagonism of the iodoacetate inhibition, but shows only that iodoacetate does not inhibit the oxidation of these added substrates as readily as the endogenous or glucose respiration. Since little quantitative information can be obtained from such studies, they will be summarized very briefly. They are mainly of historical interest.

Krebs (1931) reported that 0.3 mM iodoacetate inhibits anaerobic glycolysis and respiration markedly, and that the addition of lactate reduces the inhibition (see accompanying tabulation). Tartrate, citrate, β-hydroxybutyrate, and glycine do not alter the respiratory inhibition. However, it should be noted that some inhibition remained, especially in the tumor tissue, but whether this indicates depression of lactate oxidation is not

<table>
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<th>Tissue</th>
<th>% Inhibition of:</th>
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<tr>
<td></td>
<td>Anaerobic glycolysis</td>
</tr>
<tr>
<td>Rat testis</td>
<td>93</td>
</tr>
<tr>
<td>Rat brain</td>
<td>92</td>
</tr>
<tr>
<td>Jensen rat sarcoma</td>
<td>93</td>
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clear. Similarly, addition of lactate to iodoacetate-poisoned nerves reduces the respiratory inhibition but never completely (see accompanying tabulation) (Chang and Gerard, 1933), and in frog sartorius lactate will also par-

<table>
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<th>Iodoacetate (mM)</th>
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<th></th>
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<td>25</td>
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</tr>
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<td>80</td>
<td>65</td>
<td>65</td>
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</tr>
<tr>
<td>67</td>
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<td>70</td>
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</table>

tially relieve iodoacetate inhibition, although succinate will not (Chang and Yu, 1939). The respiratory CO₂ of rat liver slices with trilaurin as substrate is depressed 53% by 5 mM iodoacetate, but only 23% when pyruvate is added (Geyer et al., 1950 a), but this shows only that pyruvate can produce some CO₂ in the presence of iodoacetate. The respiration of beef kidney cell suspensions is inhibited moderately by 1 mM iodoacetate and pyruvate does not overcome this at all, which led Polatnick and Bachrach (1960) to conclude that the depression of the respiration is not caused by a lack of pyruvate arising from the EM pathway, this presumably meaning that iodoacetate acts elsewhere. Pyruvate also does not prevent the respiratory inhibition by iodoacetate in the fucalean alga Hormosira banksii, leading Bergquist (1958) to propose that iodoacetate, even at 0.1 mM, acts on enzymes other than 3-PGDH. On the other hand, acetate counteracts quite effectively the depression of yeast respiration by 1 mM iodoacetate, so that Schmid (1958) could assume a selective action on the EM pathway. The results from this type of experiment are thus quite variable and there is much variation between tissues.

Oxidation of Pyruvate, Lactate, and Acetate

The incorporation of pyruvate into the cycle involves lipoate, coenzyme A, and an SH-containing apodehydrogenase, so that some inhibition by iodoacetate would be anticipated. Pyruvate oxidase preparations are indeed inhibited (Table 1-13) but the data are inadequate to assess accurately the sensitivity. One can conclude only that either the enzyme from different sources is quite variable in its susceptibility to iodoacetate, or the different experimental conditions used are responsible for the variable results. The marked sensitivity of brain pyruvate oxidase observed by Peters and Wakelin (1946) led them to place it between 3-PGDH and succinate dehydro-
genase with respect to iodoacetate inhibition, and perhaps nearly equivalent to 3-PGDH in susceptibility to iodoacetamide. The degree of inhibition probably depends on the method of determining the activity: the greatest inhibition is probably observed when O₂ uptake or methylene blue reduction is measured, less when ferricyanide is the acceptor, and least when CO₂ evolution is determined. Pyruvate decarboxylase, which does not depend on the SH cofactors, may be less sensitive to iodoacetate, but the reported results (Table 1-13) are too variable for general conclusions to be drawn.

For the purpose of evaluating the sensitivity of pyruvate oxidation to iodoacetate, the most significant work is that in which the inhibition is measured of the oxygen uptake resulting from pyruvate addition to tissues. However, one must remember that the inhibition here need not be directly on the conversion of pyruvate to acetyl-CoA but could result from suppressing any step in the cycle. Inhibition of lactate respiration by iodoacetate at 1 mM or less may be confidently attributed to an effect on the metabolism of the pyruvate formed, since lactate dehydrogenase has been seen to be relatively insensitive. The moderate inhibition of pyruvate fermentation in yeast by iodoacetate around 1 mM (30-40%) reported by Yamasaki (1930) and Jensen (1931) shows that it is much less sensitive than glucose fermentation, but yet that some inhibition can be exerted in the intact cell, probably on pyruvate decarboxylase. Meyerhof and Boyland (1931) demonstrated that frog muscle treated for 1 hr with 0.25 mM iodoacetate (this inhibiting glycolysis almost completely) can oxidize lactate and pyruvate as well as normal muscle, and Lundsgaard (1932) stated that iodoacetate-poisoned yeast can oxidize pyruvate much better than glucose, although no data were given to indicate whether there is any inhibition at all. On the other hand, Smythe (1938) found that the respiration in yeast due to pyruvate is 90% inhibited by 2.1 mM iodoacetate at pH 2.5, the utilization of pyruvate being reduced to the anaerobic level. At this pH the internal iodoacetate concentration must have been much higher than in the usual experiments so that these results are perhaps not significant. The oxidation of both lactate and pyruvate in guinea pig brain brei is inhibited around 40% by 0.25 mM iodoacetate (Quastel and Wheatley, 1932); although this is less than the inhibition of glucose oxidation (90%), it is certainly appreciable. Very similar results were reported by Peters et al. (1935) in pigeon brain treated with 0.54 mM iodoacetate, and Cohen and Gerard (1937) found 92% inhibition of lactate oxidation in rat brain mince by 10 mM iodoacetate. The oxidation of lactate and pyruvate by Corynebacterium diphtheriae is inhibited 30-40% by 1 mM iodoacetate (Fujita and Kodama, 1934). This early work is thus fairly consistent in showing that iodoacetate between 0.25 and 1 mM can inhibit pyruvate oxidation in a variety of cells by 30-40%, and yet iodoacetate has been commonly con-
<table>
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<tr>
<th>Enzyme</th>
<th>Source</th>
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<th>% Inhibition</th>
<th>Reference</th>
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<td></td>
<td></td>
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<td></td>
<td>1</td>
<td>12</td>
<td>Krebs and Eggleston (1944)</td>
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<td>1</td>
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<td>50</td>
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<td>Species</td>
<td>K (mM)</td>
<td>V (umol/min/mg)</td>
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<td>van Heyningen and Pirie (1953)</td>
</tr>
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<td>Dixon <em>et al.</em> (1960)</td>
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<tr>
<td>Enzyme</td>
<td>Source</td>
<td>Reference</td>
<td>Iodoacetate (mJ)</td>
<td>% Inhibition</td>
</tr>
<tr>
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<td>Stoppani et al. (1953)</td>
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<td>Baer (1948)</td>
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<td></td>
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<td>Stampf et al. (1950)</td>
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<td>Kurtoni (1950)</td>
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<td></td>
<td>Peters and Wakelin (1946)</td>
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<td></td>
<td>Peters and Wakelin (1949)</td>
<td></td>
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<tr>
<td>Pyruvate decarboxylase</td>
<td>Escherichia coli</td>
<td>Goldfarb and Singer (1948)</td>
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<tr>
<td></td>
<td></td>
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<td>50</td>
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</tr>
</tbody>
</table>

* Iodoacetamide.
sidered since 1940 as a specific inhibitor of the EM pathway in this concentration range.

The more recent work has also been consistent in establishing that pyruvate oxidation is reasonably sensitive to iodoacetate. It is necessary to present these results briefly because this is one of the most important problems in the use of iodoacetate and every effort must be made to determine the maximal concentration which may be used without significantly depressing pyruvate oxidation, since without this information it is impossible to use iodoacetate as a selective inhibitor of the EM pathway. The oxidation of pyruvate, lactate, and acetate is inhibited 100% by 1 mM iodoacetate in *Penicillium chrysogenum*, and even 0.1 mM inhibits acetate oxidation 80% (Hockenhull et al., 1954 b). The metabolism of pyruvate by yeast is inhibited 92% by 0.58 mM iodoacetate and 96% by 1.2 mM iodoacetate whether O₂ uptake, CO₂ formation, or pyruvate utilization is measured over 180 min (Runnström and Brandt, 1941). The inhibition develops rather slowly and is not very marked during the first hour. It was concluded that iodoacetate cannot separate the oxidation and decarboxylation of pyruvate.

The uptake of pyruvate by *Chlorella ellipsoida* is more sensitive than the respiration to iodoacetate (see accompanying tabulation) (Oaks, 1962). A concentration of iodoacetate which abolishes glucose respiration does not affect pyruvate respiration, but inhibits the pyruvate uptake and the ratio of C¹⁴O₂ obtained from pyruvate-1-C¹⁴ and pyruvate-3-C¹⁴. The cells were treated 2 hr with iodoacetate. The O₂ uptake due to pyruvate in barley roots is inhibited progressively by 0.04 mM iodoacetate, the degree of inhibition depending on the time and the pH (see page 1-724) (Lattes, 1949 a). If pyruvate is added with the iodoacetate it increases the respiration strongly, but if added at varying times after the addition of iodoacetate the respiratory stimulation becomes progressively less. It was postulated that perhaps pyruvate can protect pyruvate oxidase.

The results in animal tissues are quite comparable to those found in microorganisms and plants. Marshall (1948) showed that the respiration of *Plasmodium*-parasitized chick erythrocytes in the presence of pyruvate or lactate is inhibited 75–85% by 0.9 mM iodoacetate. Webb *et al.* (1949)
observed that 1 mM iodoacetate completely prevents the respiratory stimulation by pyruvate in rat ventricle slices. The most complete study of the heart was done by Miller and Olson (1954), using duck ventricle slices. The results are similar to those in *Chlorella*, in that the uptake of pyruvate and lactate is quite sensitive, being depressed by 0.01 mM or lower iodoacetate, whereas the respiration is inhibited by concentrations around 1 mM (Fig. 1-9). Since the uptake of pyruvate can be inhibited 66% before an effect on respiration is observed, the authors suggested that another pathway for pyruvate metabolism, more sensitive than respiration to iodoacetate, is operative. This might be a reversal of the E.M pathway toward 3-phosphoglycereraldehyde. Such an explanation may well apply to *Chlorella*. The oxidation of pyruvate by a rat brain suspension is gradually depressed by iodoacetate: 0.27 mM inhibits little during the first hour but after 5 hr the inhibition is nearly complete, while 3.2 mM inhibits completely in 2 hr, the effect on lactate oxidation being very similar (Bernheim and Bernheim, 1941). In electrically stimulated guinea pig brain slices, the O$_2$ uptake with lactate as the substrate is not inhibited by iodoacetate until a concentration of around 0.08 mM is reached, whereas the respiration with glucose is already depressed at 0.01 mM (Heald, 1953). Thus in this tissue, although lactate oxidation is quite sensitive to iodoacetate, it would be possible to
achieve a reasonably selective block of the EM pathway. It may be noted that the respiration of unstimulated slices with either glucose or lactate is not appreciably affected by iodoacetate up to 0.1 mM.

The only thorough study of the effects of iodoacetate on the cycle in isolated mitochondria is that of Yang (1957). Rat heart mitochondria were incubated with the inhibitors for 10 min and the respiration measured over 1 hr after adding the various substrates (Table 1-14). There is a very sub-

Table 1-14

<table>
<thead>
<tr>
<th>Substrate</th>
<th>% Change</th>
<th></th>
<th></th>
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</thead>
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<tr>
<td></td>
<td></td>
<td>Iodoacetate</td>
<td></td>
<td>Iodoacetamide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01 mM</td>
<td>0.1 mM</td>
<td>1 mM</td>
</tr>
<tr>
<td>Pyruvate + malate</td>
<td>- 4.3</td>
<td>- 43.0</td>
<td>- 85.6</td>
<td>- 9.4</td>
</tr>
<tr>
<td>Citrate</td>
<td>+ 4.6</td>
<td>- 8.1</td>
<td>- 34.6</td>
<td>-16.1</td>
</tr>
<tr>
<td>Isocitrate</td>
<td>+15.0</td>
<td>-15.3</td>
<td>-35.0</td>
<td>-12.0</td>
</tr>
<tr>
<td>a-Ketoglutarate</td>
<td>- 6.3</td>
<td>-33.3</td>
<td>-75.6</td>
<td>- 6.4</td>
</tr>
<tr>
<td>Succinate</td>
<td>- 3.8</td>
<td>- 8.0</td>
<td>-61.2</td>
<td>- 2.5</td>
</tr>
<tr>
<td>Malate</td>
<td>- 7.4</td>
<td>-20.0</td>
<td>-63.9</td>
<td>- 1.0</td>
</tr>
</tbody>
</table>

*From Yang (1957).*

stantial inhibition of pyruvate oxidation by 0.1 mM iodoacetate, but only slight effects at 0.01 mM. Iodoacetate at 1 mM suppresses all oxidations quite strongly. Although the cycle is not as readily inhibited as the EM pathway, such results should deter investigators from glibly terming iodoacetate a specific inhibitor of glycolysis, especially after using concentrations of 1 mM or over.

The effects of iodoacetate on the oxidation of acetate also afford evidence for the inhibition spectrum. In *Pseudomonas calco-acetica* proliferating aerobically in an acetate medium, some of the acetate is assimilated into cell material and some is oxidized. Iodoacetate above 0.0125 mM begins to reduce the rate of oxidation, but at 0.014–0.1 mM increases the fraction of the acetate oxidized; i.e., it inhibits assimilation more than oxidation (Clifton, 1937). The oxidation of acetate by *Corynebacterium creatinovorans* (Singer and Barron, 1945) and *Acetobacter peroxidans* (Tanenbaum, 1955) is inhibited over 90% by 5 mM iodoacetamide and 0.5 mM iodoacetate, respectively. Iodoacetate at 1 mM blocks fermentation and glucose respira-
tion completely in yeast, but when acetate is added the inhibition is much reduced (Schmid, 1958). This was taken as evidence that iodoacetate inhibits glycolysis selectively. Essentially the same was seen in *Alloomyces macrogynus* by Bonner and Machlis (1957), 1 mM iodoacetate inhibiting glucose respiration 90% and acetate respiration 29%, while 0.1–0.5 mM inhibits glucose respiration without significant effect on acetate oxidation, and this was again used as evidence for a specific block of 3-PGDH, acetate bypassing this block. However, it must be realized that acetate also bypasses pyruvate oxidase, and acetate kinase is apparently quite resistant (Table 1-13), so that the results with acetate cannot be extrapolated to the normal situation in which pyruvate is the major substrate for the cycle.

The inhibition of pyruvate oxidation could be due to inactivation of coenzyme A or lipoate. Shimi and Nour El Dein (1962), for example, postulated that the accumulation of certain cycle intermediates in *Aspergillus terreus* treated with iodoacetate at 0.01 mM might be due to reaction with coenzyme A. The initial work of Lynen *et al.* (1951) has often been taken as indicating that coenzyme A is readily inactivated by iodoacetate, but the conditions of the experiment have usually been overlooked. The reduced coenzyme A was incubated with 33 mM iodoacetate at pH 7 and room temperature for 2 hr; less than 5% of the coenzyme A activity remained as tested in an acetylation reaction. Whether significant reaction would take place with iodoacetate at 1 mM or below is not known, and experiments along this line with both coenzyme A and lipoate are needed. It requires 17 mM iodoacetate to reduce the coenzyme A activity in yeast by 25%, and 43 mM to reduce it 50% (Estler *et al*., 1960). It has been shown by Davenport *et al.* (1956) that mouse stomach incubated with iodoacetamide exhibits no reduction of coenzyme A activity over 10 min until a concentration of 8 mM is reached (−30%), and that no inactivation of lipoate occurs with 5 mM iodoacetamide over 30 min. These results indicate that within tissues the inhibitions brought about by iodoacetate and iodoacetamide are not related to carboxymethylation of these cofactors, and that the inhibition of pyruvate oxidation is probably through reaction with SH groups on the apodehydrogenase.

Summarizing the situation with respect to the relative inhibitions of the EM pathway and pyruvate oxidation, one can safely state that it is probably impossible to obtain a completely selective action on the EM pathway (i.e., 100% depression of pyruvate formation without an effect on pyruvate oxidation), and that in general it is very difficult to achieve marked inhibition of the EM pathway without to some extent suppressing pyruvate oxidation. The problem is complicated by the variable sensitivities of the two systems in different organisms and tissues; in some cells it is likely that much greater specificity can be obtained than in others. Another important factor is time. The EM pathway seems to be blocked more rapidly
than pyruvate oxidation by iodoacetate, so one can visualize a reasonably selective action over 10–30 min if the proper concentration is chosen, with the selectivity decreasing with time. There is one fact which is strongly established: when iodoacetate or iodoacetamide is used at 1 mM or above, there can be no selective block of the EM pathway. Indeed, in most cases concentrations well below 1 mM are by no means selective. These concentrations are those in the regions of 3-PGDH and the cycle. In cellular preparations the external concentration in the medium may be higher than within the cell, and this will vary greatly with the permeability properties of the cells and the pH, but most of the work discussed in this section indicates that iodoacetate inhibits to some extent the oxidation of pyruvate in cells when present in concentrations between 0.1 and 1 mM in the medium. If a selective EM pathway block in a particular tissue is desired, it is necessary to examine the effects of different concentrations of iodoacetate on glycolysis and pyruvate oxidation so as to obtain evidence that under the particular conditions such a block can be achieved. Lower concentrations than are usually used would be expected to be more effective. I believe that it is possible in most cells to obtain an inhibition of the EM pathway which is selective enough for relating this pathway to some cell activity, but to do this requires the proper preliminary experiments to establish the optimal conditions. This matter will be taken up again when the effects of iodoacetate on cellular function are discussed.

General Effects on the Cycle and Mitochondria

The data presented in Tables 1-13 and 1-14 suggest that several steps in the cycle, in addition to the initial reaction of pyruvate, are sensitive to iodoacetate, but the results are often so inconsistent that it is difficult to evaluate the importance of these inhibitions. α-Ketoglutarate oxidase is perhaps as sensitive as pyruvate oxidase, while isocitrate dehydrogenase, succinate oxidase, and malate dehydrogenase are moderately inhibited. The over-all effect of these simultaneous inhibitions on the cycle may be greater than any single inhibition, but there have been no studies of these over-all effects in isolated mitochondria. Several reports on citrate levels as influenced by iodoacetate have been made but are difficult to interpret. Iodoacetate does not act like fluorocacetate as far as is known and does not form an inhibitory iodocitrate. Kalnitsky (1948) showed in kidney homogenates that only fluorocacetate of all the haloacetates leads to citrate accumulation. One would indeed expect iodoacetate to decrease citrate levels, due to either a reduction in the formation of pyruvate or the utilization of pyruvate, and such has been found in mammary gland by Terner (1955). However, Gal et al. (1956) reported some citrate accumulation in the kidneys of rats given 1.8 mg/kg iodoacetate (± 65%) while citrate decreases in the liver (− 37%), but only two animals were used. Citrate fermentation in Aspergillus niger
is strongly inhibited by iodoacetate but this is probably due to a block of the EM pathway (Damodaran and Bangachari, 1951), while the moderate inhibition of citrate formation anaerobically in rat testis is presumably related to an action on the formation of acetyl-CoA (Paul et al., 1954).

The inhibition of the endogenous respiration of rat liver mitochondria by iodoacetate (25% by 0.2 mM and 70% by 1 mM) may be taken as evidence that iodoacetate exerts a definite action on the cycle (Minnaert, 1960), but it is also possible, not knowing the nature of the endogenous substrate, that part of the inhibition is on a pathway leading to the cycle (e.g., the fatty acid helix). Another effect of iodoacetamide on mitochondria must be considered, namely, the marked swelling produced by concentrations of 5–10 mM (Tapley, 1956; Hunter, 1961). Since other SH reagents produce swelling, it has been postulated that SH groups are involved in the mitochondrial structure, but if so this is probably mediated through metabolic disturbances. Whether such effects occur with lower concentrations of these alkylating agents is not known.

PHOSPHORUS METABOLISM

One more aspect of metabolism must be considered before approaching the effects of iodoacetate on carbohydrate oxidation, namely, the changes in the various phosphate fractions, inasmuch as this is intimately connected with the control of carbohydrate metabolism. One would expect iodoacetate to depress the uptake of P4 in synthesizing and growing cells, since the formation of ATP and consequently the synthesis of nucleotides, nucleic acids, phospholipids, and other phosphorus-containing substances would be inhibited. We have seen that the hexose mono- and diphosphates may increase in response to iodoacetate (although even their formation will be eventually stopped), but these are probably the only phosphates accumulating. Inhibition of P4 uptake (i.e., total net phosphorylation) by iodoacetate or iodoacetamide has been demonstrated in *Micrococcus aureus* (Hotchkiss, 1956), yeast (Kamen and Spiegelman, 1948; Hoffmann-Ostenhof and Kriz, 1950; Stickland, 1956 c), sea urchin eggs (Lindberg and Ernstner, 1948), human erythrocytes (Gourley, 1951), intestine (Naitô, 1944), rabbit lens (Müller and Kleifeld, 1953), rat thymus nuclei (Ord and Stocken, 1961), and particulate suspensions of rat liver, brain, and tumor (Clowes and Keltch, 1952). In most cases this is probably due to an inhibition of the EM pathway. Iodoacetate actually causes a loss of cell P4 under certain conditions. The changes in P4 may be quite complex because of the many phosphorylated compounds and the multitude of reactions involving phosphate (e.g., oxidative phosphorylation, ATPases, phosphatases, transphosphorylases, and various phosphorylations), even though the inhibition is only on 3-PGDH. The changes will often depend on the initial state of the tissue when iodo-
acetate is added. For example, if Ehrlich ascites cells are incubated in nitrogen for 1 hr at 37° the P, level is high; when glucose is added there is a rapid decrease in P, (Laws and Stickland, 1962). Iodoacetate at 1 mM completely prevents this fall in P, anaerobically; no hexose phosphates can be formed because there is no ATP. Aerobically the inhibition is 75%, indicating other pathways for phosphorylation. When ATP is initially high, or is added, P, will decrease in the presence of iodoacetate.

The uptake of phosphate by cells may not be entirely a simple inward diffusion to replace intracellular phosphate incorporated metabolically. A very interesting study of phosphate intake by the yeast cell was made by Leggett (1961) on the basis of a carrier mechanism in the membrane. The uptake is coupled with the synthesis of ATP associated mainly with the 3-PGDH reaction; one component is rate-limited by the phosphorylation of ADP and a second by the hexokinase reaction. A third minor component is associated with the oxidation of cytochrome b. Inasmuch as the phosphate uptake is related predominantly to 3-PGDH activity, it is not surprising that iodoacetate readily inhibits it. Leggett claims that iodoacetate reduces the concentration of a carrier-P complex and inhibits phosphate uptake uncompetitively (since the maximal uptake rate is changed and K_m is not, the inhibition must be classed formally as noncompetitive, although what this means kinetically is difficult to say). Unfortunately, Leggett used 10 mM iodoacetate so that any interpretation of the results based on a selective glycolytic action must be accepted cautiously.

The most important aspect of phosphorus metabolism for our purpose is the levels of adenine nucleotides and creatine-P within cells and the effects of iodoacetate upon them. Synthesis of ATP should be depressed aerobically and to a degree dependent on the activity of iodoacetate-resistant pathways for oxidative phosphorylation. Any uncoupling action exerted by iodoacetate (see page 61) will also be of importance. Early workers showed decreases in the creatine-P of nerves and muscle (Gerard and Tupikow, 1931) and the heart (Burns and Cruickshank, 1937) following treatment with iodoacetate, which is probably a consequence of the fall in ATP. Decreases in cellular ATP levels have been reported in Aspergillus niger (Mann, 1944), human erythrocytes (Prankerd and Altman, 1954), intestinal mucosa (Fehér et al., 1956), calf lens (Nordmann et al., 1954), guinea pig seminal vesicle mucosa (Whittam and Breuer, 1959), and Ehrlich ascites cells (Thomson et al., 1960). In most cases rather marked effects have been observed at concentrations of iodoacetate around 1 mM. In some instances the fall in ATP is accelerated by its utilization, as in the formation of hexose phosphates in brain extracts (see tabulation on page 75) (Geiger, 1940), or by cell activity, or by ATPases. The effect of any inhibitor on ATP level will depend on the balance of synthesis and breakdown. Iodoacetate produces primarily an inhibition of synthesis, as shown by Nakao et al. (1960) in erythrocytes,
where iodoacetate at 1 \( M \) completely blocks ATP synthesis from adenine and inosine.

Turning to studies in which the patterns of nucleotide change have been recorded, a good deal of variability in response is noted (Table 1-15) as might be expected on the basis of the different metabolic pathways for ATP generation available in the organisms. The effects of iodoacetate in reducing ATP and creatine-P levels are more marked under anaerobic than aerobic conditions, and more marked in the presence of glucose than endogenously, and occur faster if the tissue is functionally active. The rates of change of these nucleotides following iodoacetate inhibition have seldom been measured, but in *Chlorella* (Fig. 1-7) and erythrocytes (Fig. 1-8) the changes with time are quite as anticipated. The initial rise in ADP is probably common and is due to the first reaction in the sequence ATP \( \rightarrow \) ADP \( \rightarrow \) AMP being initially relatively fast (when ATP falls sufficiently the rate falls).

The very marked metabolic difference between avian and mammalian erythrocytes is nowhere better seen than in the response to iodoacetate (Table 1-15). The essentially glycolytic ATP generation in human erythrocytes is readily inhibited by iodoacetate, whereas pigeon erythrocytes apparently can maintain their ATP levels aerobically in the presence of glucose by alternate pathways. The greater effects of iodoacetate on contracting muscle point out the importance of functional activity in a tissue in determining the response to inhibitors, and this must always be taken into account in working with active tissues such as the heart or proliferating organisms. When a frog muscle goes into contracture induced by bromoacetate, the pattern of the phosphate compounds changes markedly (see accompanying tabulation) (Hermans, 1956). These changes not only are

<table>
<thead>
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<th>Compound</th>
<th>Concentration in muscle</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Resting</td>
<td>Contracture</td>
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<tr>
<td>ATP</td>
<td>324</td>
<td>138</td>
</tr>
<tr>
<td>ADP</td>
<td>89</td>
<td>54</td>
</tr>
<tr>
<td>( P_i )</td>
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<td>275</td>
</tr>
<tr>
<td>CrP</td>
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<td>119</td>
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<tr>
<td>Hexose-P's</td>
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<td>44</td>
</tr>
<tr>
<td>Hexose-diP</td>
<td>30</td>
<td>434</td>
</tr>
</tbody>
</table>

of significance in interpreting the secondary metabolic effects of iodoacetate, but also must be an important factor in the development of the contracture.
The effects of iodoacetate on carbohydrate oxidation and respiration are generally very complex, and the failure to appreciate the various factors involved has given rise to erroneous theories and conflicting concepts. We shall be particularly concerned with the effects of iodoacetate on aerobic glycolysis, the degree and pattern of glucose utilization, respiration, and the alternate pathways of carbohydrate metabolism. Iodoacetate has been useful in the detection of routes other than the EM pathway, and it will be especially important to determine the role these alternate routes play in the oxidation of carbohydrates.

**Aerobic Glycolysis**

Aerobic glycolysis as measured by the formation of lactate should be inhibited by iodoacetate but the degree of inhibition need not be the same as for anaerobic glycolysis, although such seems to have been frequently assumed. Anaerobic glycolysis is usually inhibited somewhat more effectively than aerobic glycolysis: this is true in guinea pig lymph node cells, mammary gland, rat brain, HeLa cells, and Ehrlich ascites cells (see Table 1-16 for references). Crabtree and Cramer (1933 a) found no significant difference in rat sarcoma, and Villavicencio and Barron (1957) found aerobic glycolysis to be the more sensitive in mouse lymphosarcoma. Aerobic glycolysis is not a very important measurement and is more often a measure of the unphysiological conditions under which the tissue is placed than an indicator of metabolism. One might conceive of aerobic glycolysis as an expression of the thermodynamic inefficiency of a tissue (the fraction of glucose going to lactate compared to that oxidized through the cycle would be better), and this is usually increased when a tissue is hypoxic or damaged. Nevertheless, the effects of iodoacetate on aerobic glycolysis are of some interest, especially in illustrating the inhibition of a multienzyme system.

Aerobic glycolysis in the simplest case can be represented by a divergent chain:

\[
\begin{align*}
\text{Glucose} & \xrightarrow{(1)} \text{pyruvate} \\
& \xrightarrow{(2)} \text{acetyl-CoA} \xrightarrow{\text{cycle}} \\
& \xrightarrow{(3)} \text{lactate}
\end{align*}
\]

The rate of lactate formation will depend on the relative rates of reactions (2) and (3), as well as on the rate of the EM pathway. If reactions (2) and (3) are kinetically equivalent, the inhibition of both will be the same as an inhibition exerted on pathway (1), but this is usually not the case and
### Table 1-15

**Effects of Iodoacetate on the Levels of Adenine Nucleotides and Creatine-P**

<table>
<thead>
<tr>
<th>Organism or tissue</th>
<th>Conditions</th>
<th>Compound</th>
<th>Iodoacetate (mM)</th>
<th>% Change</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Physarum polycephalum</em></td>
<td>Aerobic</td>
<td>ATP</td>
<td>0.67</td>
<td>+ 3</td>
<td>Hatano and Takeuchi (1960)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ADP</td>
<td>0.67</td>
<td>+ 19</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P_i</td>
<td>0.67</td>
<td>+ 25</td>
<td></td>
</tr>
<tr>
<td>Pigeon erythrocytes</td>
<td>Aerobic</td>
<td>ATP</td>
<td>0.5</td>
<td>- 2</td>
<td>Gerlach and Lübben (1959)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ADP</td>
<td></td>
<td>- 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>AMP</td>
<td></td>
<td>+ 3</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>GTP</td>
<td></td>
<td>+ 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GDP</td>
<td></td>
<td>+ 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>P_i</td>
<td></td>
<td>+ 1</td>
<td></td>
</tr>
<tr>
<td>Human erythrocytes</td>
<td>Aerobic</td>
<td>ATP</td>
<td>0.5</td>
<td>- 89</td>
<td>Gerlach and Lübben (1959)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ADP</td>
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<td>0.7$^b$</td>
<td>El'tsina and Beresotskaya (1962)</td>
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<tr>
<td>With glucose</td>
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<td>ATP</td>
<td>0.7$^b$</td>
<td>El'tsina and Beresotskaya (1962)</td>
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<td></td>
<td>P</td>
<td>-58</td>
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</table>

$^a$ Easily hydrolyzable phosphate taken as mainly ATP.

$^b$ Bromoacetate.

$^c$ These results are probably not quantitative since there was only one iodoacetate control; the differences are perhaps more related to normal tissue variations, but tend to show little effect of iodoacetate.

$^d$ These results do not show the total nucleotide levels but the inhibition of the incorporation of $P_i^{32}$ into the nucleotides.

$^e$ Control AMP was zero so that appearance of AMP (which in iodoacetate-treated cells is higher than either ADP or ATP) cannot be expressed readily as a per cent.
## Inhibition of Aerobic Glycolysis by Iodoacetate

<table>
<thead>
<tr>
<th>Organism and tissue</th>
<th>Iodoacetate (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
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<tr>
<td>Guinea pig polymorphonuclear leucocytes</td>
<td>0.1</td>
<td>60</td>
<td>Sbarra and Karnovsky (1959)</td>
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<tr>
<td>Rabbit lymphatic cells</td>
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<td>Villavicencio and Barron (1957)</td>
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<tr>
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<tr>
<td></td>
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<td>99</td>
<td></td>
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<tr>
<td>Guinea pig lymph node cells</td>
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<td>98</td>
<td>Helmreich and Eisen (1959)</td>
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<td>Bull spermatozoa</td>
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<td>Lardy and Phillips (1943 b)</td>
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<td>68</td>
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<tr>
<td>Whole</td>
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<td>99</td>
<td>Futterman and Kinoshita (1959)</td>
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<tr>
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<td>87</td>
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<tr>
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<td>0.1</td>
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<td>Wu and Racker (1959)</td>
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thus aerobic glycolysis will be inhibited more or less than the formation of pyruvate. Reference should be made to the discussion of the inhibition of divergent chains in Chapter I-7. If we simplify the problem by assuming that reactions (2) and (3) follow ordinary enzyme kinetics:

\[ v_1 = \frac{V_2 \text{ (pyr)}}{K_2 + \text{ (pyr)}} + \frac{V_3 \text{ (pyr)}}{K_3 + \text{ (pyr)}} \]

where the \( K \)'s and \( V \)'s are the Michaelis constants and maximal rates for each of the reactions. Analysis of such a system shows that the reaction which has the lower potential rate will be inhibited less than \( v_1 \). In the above formulation we have neglected other reactants. In reaction (3), NADH also must be considered. It is likely that aerobically the concentration of NADH (or the NADH/NAD ratio) will be less than aerobically, and hence that reaction (3) will be slower than might be anticipated from anaerobic glycolysis rates. From this and other considerations one might predict that reaction (2) would be potentially greater than reaction (3). To illustrate the behavior of such a system, let us assume \( V_2 = 50 \), \( V_3 = 10 \), \( K_2 = 0.2 \text{ mM} \), and \( K_3 = 0.1 \text{ mM} \), and calculate the rates of all the reactions in a steady state. It is further assumed that the uninhibited rate of reaction (1) is near maximal and iodoacetate inhibits this 57%. We find that pyruvate oxidation is inhibited 60% and aerobic glycolysis only 45% (see tabulation).

<table>
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<th>Uninhibited system</th>
<th>Inhibited system</th>
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<td>( v_1 )</td>
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</tr>
<tr>
<td>( v_2 )</td>
<td>41.6</td>
</tr>
<tr>
<td>( v_3 )</td>
<td>9.1</td>
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Another factor which can markedly reduce the effect of iodoacetate on aerobic glycolysis is an inhibition of reaction (2). This essentially increases the pyruvate concentration and shifts the metabolism to lactate. In the example above, if reaction (2) is inhibited only 10% by iodoacetate, the inhibition on aerobic glycolysis will drop to 26%; if the inhibition of reaction (2) is 20%, the effect on lactate formation almost disappears, and if the inhibition of reaction (2) is greater than this, even stimulation of aerobic glycolysis is possible. Since iodoacetate can somewhat depress pyruvate oxidation at concentrations blocking the EM pathway, such an effect should be fairly common. The stimulation of aerobic lactate formation in rat thymocytes by low concentrations of iodoacetamide (slight stimulation at 0.01 mM and maximal stimulation at 0.1 mM) with inhibition at higher concentrations indicates that the EM pathway is not the most sensitive system here (Araki and Myers, 1963).
Actually it is more accurate to think of lactate simply in equilibrium with one intermediate in the aerobic oxidation of glucose, namely, pyruvate, just as dihydroxyacetone-P is in equilibrium with 3-phosphoglyceraldehyde. The concentration of lactate in a tissue will fluctuate with the pyruvate concentration and an inhibitor affecting the formation or disposal of pyruvate will modify its level. In addition, the NADH/NAD ratio is important in this equilibrium. One would expect that the steady-state level of lactate would be reached quite rapidly in a tissue and remain there as long as the rate of glucose oxidation is constant. Measurements of aerobic glycolysis really involve determinations of steady-state lactate concentrations in most cases. An increase over a period in the lactate level is often interpreted as aerobic glycolysis, whereas such increase is probably due to changes in the tissue leading to impaired cycle operation. Looked at in this way, the effects of iodoacetate fall into the category of pseudosteady-state modifications in a multienzyme system.

The effect of iodoacetate on tissue lactate in vivo, such as Lundsgaard’s early observations with muscle, is basically a matter of aerobic glycolysis. No study has been made of the comparative effects of iodoacetate on different tissues after injection into animals, but reduction of lactate occurs in the brain and kidney of dogs given iodoacetate, which is allowed to incubate for 10 min (Haldi, 1932). In kidney, lactate formation is essentially abolished; in the brain, the results are erratic but somewhat over 50% depression seems to be the rule. There is thus evidence that iodoacetate can produce changes in intact tissue metabolism similar to those seen in slices or extracts.

The effects of iodoacetate on lactate formation and respiration of brain slices are very interesting and present some unexplainable phenomena (Heald, 1953). The results are summarized in Fig. 1-10. Three things are immediately evident: (1) aerobic glycolysis is inhibited more than respiration, (2) electrically stimulated slices are inhibited more than resting slices, and (3) the increase in inhibition with iodoacetate concentration is complex. The first point will be discussed later (page 122). Stimulation increases aerobic glycolysis some 50% and iodoacetate reduces this extra lactate formation. Stimulation apparently increases the contribution of glucose to O₂ uptake, so the greater inhibition of stimulated slices is not surprising. The shapes of the curves are not so easy to explain. It would appear offhand that more than one reaction is inhibited, the first being completely inhibited by 0.01 mM iodoacetate and the second being inhibited only above 0.04 mM. These might be 3-PGDH and the hexokinase reaction. However, in such a complex multienzyme system one must beware of facile interpretations. Another explanation might be the following. At the high glycolytic rate of stimulated tissue the oxidation of pyruvate is saturated, i.e., the pyruvate concentration is high enough so that its reduction by partial inhibition of the EM pathway does not reduce its oxidation, which is substan-
tiated by the low respiratory inhibition at 0.01 mM. As the iodoacetate concentration is increased, the EM pathway is depressed more, and the pyruvate concentration reaches the level where it becomes limiting to its oxidation; at this point respiration is inhibited, as seen by the sudden rise in inhibition at 0.01-0.04 mM iodoacetate. There may also be some slight direct inhibition of pyruvate oxidation so that the concentrations of pyruvate and lactate do not change significantly. Finally, when the iodoacetate concentration is such as to block the EM pathway essentially completely, the lactate concentration falls again. Other equally tenous explanations could be easily adduced.

Fig. 1-10. Effects of iodoacetate on aerobic glycolysis and respiration of guinea pig brain slices. (Data from Heald, 1953.)

Endogenous Respiration

The effects of iodoacetate on the endogenous respiration provide little information on either the site of iodoacetate action or the nature of this respiration, although occasionally some indication of the principal endogenous substrate is obtainable if the experiments are properly run. However, it is very important to determine the inhibition of the endogenous respiration if the action of iodoacetate on the oxidation of some added substrate is being examined, since correction for the endogenous effect must be made (I-441). Such a correction has been used very little in the work with iodoacetate and glucose oxidation. Selected examples of the inhibition
of endogenous respiration from a variety of organisms and tissues are given in Table 1-17. The degree of inhibition will depend on a number of factors: (a) the substrate or substrates being oxidized, (b) the sensitivity of the particular 3-PGDH or EM pathway, (c) the inhibition of pathways other than the EM pathway, (d) the permeability of the cells to iodoacetate, and the external pH, and (e) the time of exposure to iodoacetate. The latter two factors make it difficult to evaluate quantitatively the results in the table and to compare the inhibitions in different organisms or tissues. In many cases the inhibition progresses steadily with time so that a single value for inhibition is meaningless, and furthermore it is quite possible that secondary effects soon appear so that the inhibition is not solely due to the immediate action of iodoacetate. It may be seen from the table that the iodoacetate concentrations used have often been much higher than required to block the EM pathway. Although the concentration within the cells is not known, one must assume that the action is not specific on the EM pathway, since concentrations of 1 mM or even lower have been shown frequently to inhibit pyruvate oxidation quite significantly.

A few general deductions may be made from the results in Table 1-17. Although endogenous respiration is usually inhibited by iodoacetate, it is much less sensitive than anaerobic or aerobic glycolysis. If iodoacetate at 0.01-0.02 mM inhibits the EM pathway around 50% (page 63), it usually requires over 100 times this to inhibit endogenous respiration comparably, although in certain cells endogenous respiration is reasonably sensitive. The principal reason for this is probably that the endogenous respiration commonly does not depend very strongly on carbohydrate. It may also be noted that there is often an iodoacetate-resistant fraction of the endogenous respiration (i.e., the inhibition levels off even though the iodoacetate concentration is raised above 10 mM). The nature of this resistant fraction is not known, but it may consist mainly of simple oxidations of dicarboxylates and amino acids. In fact, the increasing inhibition with time may occasionally be due to the utilization and depletion of substrates oxidized through iodoacetate-resistant pathways.

Iodoacetate in common with other SH reagents stimulates the endogenous respiration of certain cells at low concentrations. This has been observed in Streptomyces griseus (Hockenhull et al., 1954 a), yeast (Lundsgaard, 1930 c), Chlorella pyrenoidosa (Kohn, 1935), the slime mold Physarum polycephalum (Allen and Price, 1950), sea urchin spermatozoa (Barron and Goldinger, 1941; Barron et al., 1948), bull spermatozoa (Lardy and Phillips, 1943 a), toad retina (Hanawa et al., 1956), cat salivary gland (Druckrey and Loch, 1943), and the Earle strain of mammalian cells in culture (Siegel and Cailleau, 1956). Sometimes this stimulation is maintained but usually it is temporary and passes over into an inhibition. The stimulation may be as much as 100%, as in Chlorella at around 0.1 mM iodoacetate (Kohn,
1935), although Kandler et al. (1961) found only inhibition, an unexplained discrepancy. Barron and Goldinger (1941) found that 1 mM iodoacetate markedly stimulates the respiration of sea urchin spermatozoa, and showed that this is not due to a splitting of the iodoacetate into acetate and iodine, since acetate does not stimulate the respiration and iodine only inhibits. The stimulation involves the whole respiration since the CO₂ output increases to the same degree, the R.Q. remaining constant. Barron et al. (1948) postulated that the stimulation may be due to the reaction of iodoacetate with the soluble thiols of the cells, thereby removing a control of respiration, whereas respiratory inhibition is due to reaction with the fixed (or enzyme) SH groups. In most of the cases of stimulation the pH was above 7, and for the sea urchin spermatozoa was 8.2. Possibly an initial action on the membranes of the cells occurs, leading to permeability changes and increased respiration. Another explanation may be related to the Crabtree effect (increased glucose depresses mitochondrial oxidations), in that blocking of the EM pathway may temporarily stimulate the oxidation of accumulated substrates through the cycle.

**Respiration in the Presence of Glucose**

One might expect that the inhibition of respiration in the presence of glucose would be greater than that of endogenous respiration on the basis that more of the O₂ uptake is dependent on the EM pathway. However, this is not necessarily true. First, the presence of iodoacetate-resistant oxidative pathways for glucose could allow glucose to be oxidized more readily than the endogenous substrates. Second, the greater rate of oxidations in the presence of glucose, even in the presence of iodoacetate, could maintain a higher ATP level and hence phosphorylation of glucose would not fail as rapidly as the endogenous respiration. Third, in certain tissues with high aerobic glycolysis, such as HeLa cells, the addition of glucose aerobically increases the lactate formation markedly but may even depress the respiration. Here iodoacetate will inhibit glucose utilization and lactate production, but the iodoacetate-resistant fraction of the respiration may remain essentially the same, resulting in a smaller per cent inhibition of the respiration in the presence of glucose. Some inhibitions of glucose respiration are presented in Table 1-18 (others will be mentioned later). Where direct comparison of endogenous and glucose respiration can be made, one finds that the latter may be inhibited more, the same, or less than the endogenous respiration, which is actually what one should expect. The accompanying tabulation shows examples of all three situations. In Ehrlich ascites tumor cells, iodoacetate at 0.1 mM almost completely prevents the formation of lactate, inhibits endogenous respiration 15%, but actually stimulates the O₂ uptake with glucose present due to the suppression of the Crabtree effect from 48% to 28% (Wenner and Cereijo-Santalo, 1962).
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<th>Concentration (mM)</th>
<th>% Inhibition</th>
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Morrison (1950)
Allen and Price (1950)
Ryley (1955 b)
Doran (1957)
Ryley (1955 a)
Ryley (1952)
Agosin and von Brand (1953)
Clowes and Krahl (1940)
Cleland and Rothschild (1952 b)
Agosin et al. (1957)
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* The inhibitors are designated as follows: IA = iodoacetate; IAM = iodoacetamide; and BrA = bromoacetate.
### Table 1-18

**Inhibition of Respiration in the Presence of Glucose by Iodoacetate**

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<td>Human</td>
<td>HeLa cells</td>
<td>IA</td>
<td>7.4</td>
<td>0.3</td>
<td>55</td>
<td>Wu (1959)</td>
</tr>
</tbody>
</table>

average of cervical carcinoma

*Inhibition of glucose oxidation corrected for endogenous inhibition, assuming that addition of glucose does not alter the basal metabolism.

b Inhibitors are designated as follows: IA = iodoacetate; IAM = iodoacetamide.
Wu (1959) pointed out that the metabolism of glycogen or glucose by HeLa cells depends on the conditions of culture and other factors, so that different steps may be limiting. This is probably true for all cells to varying degrees since these metabolic pathways are capable of a great deal of flexibility. The inhibition of glucose respiration in any particular cell or tissue is likely to vary widely with not only the experimental conditions but the past history. Examples are the greater sensitivity of the culture form compared to the blood form of *Trypanosoma rhodesiense*, 0.033 mM iodoacetate inhibiting the former 53% and the latter not at all (Ryley, 1962), and the dependency of the inhibition of brain respiration on the age of the animals (Tyler, 1942; Muir et al., 1959). The increasing sensitivity to malonate and the decreasing sensitivity to iodoacetate with increasing age of rats indicate that the metabolic patterns or controls are changing in the brain.

**Differential Effects on Glycolysis and Respiration**

Inspection of Tables 1-11, 1-17, and 1-18 shows that anaerobic glycolysis is inhibited by iodoacetate to a much greater degree than respiration. Lundsgaard (1930 c) in his initial work with yeast found that iodoacetate at 1.1 mM inhibits fermentation completely without significant effect on respiration over a period of an hour. This was termed the “Lundsgaard effect” by Turner (1937), who gave a complete summary of the early work. This differential effect was observed by a number of workers in all types of organism and tissue, although most found only a “modified” Lundsgaard effect in that respiration is depressed less or more slowly than glycolysis, and some of these investigators suggested that glycolysis and respiration are two entirely distinct metabolic phenomena. Such data seemed to argue...
against the unitary concept of glucose oxidation, wherein glucose is broken down through an anaerobic pathway to pyruvate (lactate in the early work) and then oxidized through another pathway. On the other hand, Krebs (1931) found in rat tissues that anaerobic glycolysis and glucose respiration are inhibited comparably by iodoacetate, and favored the unitary concept. Similar results were reported by Crabtree and Cramer (1933 a) on rat sarcoma, and by Fujita and Kodama (1934) on Corynebacterium diphtheriae, in both cases anaerobic glycolysis and respiration with glucose being inhibited to about the same degree. These conflicting results and the incomplete knowledge of the pathways of glucose oxidation created a confusion which has been completely resolved. We can now summarize the experimental results by saying that in most cells under the usual conditions the anaerobic glycolysis is inhibited more readily than respiration by iodoacetate, although very seldom can a completely differential effect be observed, and in some instances there may be very little difference in susceptibility. It should also be pointed out that only with a carefully chosen range of iodoacetate concentrations can significantly different inhibitions on glycolysis and respiration be obtained. Some tissues in which a very clear differential effect is observed are frog muscle (Wright, 1932; Saslow, 1936; Stannard, 1938 a), eat and dog heart (Shorr et al., 1938), rat and guinea pig brain (Heald, 1953; Lisovskaya, 1956; McMurray et al., 1957 a), and Ehrlich ascites carcinoma cells (Holzer et al., 1955 c, 1958; Maizels et al., 1958), as well as in yeast (Lundsgaard, 1930 c; Jensen, 1931; Nilsson et al., 1931; Stier and Stannard, 1936; Stickland, 1956 b).

A discussion of the reasons for the different susceptibilities of glycolysis and respiration to iodoacetate will bring out a number of the factors involved in the inhibition of carbohydrate metabolism. There is not one but several explanations for a differential effect.

(a) Oxidation of noncarbohydrate substrates. The oxidation of fats, amino acids, and other substrates may proceed in the presence of iodoacetate (providing the concentration is not too high) and may account for part of the iodoacetate-resistant respiration. A determination of the R.Q.'s for normal and poisoned cells will sometimes give information on this point (see page 133).

(b) Oxidation of intermediates from carbohydrate breakdown. We have seen that lactate and pyruvate can be oxidized appreciably in the presence of glycolysis-blocking concentrations of iodoacetate, and Lundsgaard (1932) and others have shown this to hold also for ethanol. Much of the iodoacetate-resistant respiration of yeast, for example, arises from the oxidation of ethanol formed before glycolytic inhibition is complete. Lundsgaard showed that the longer the fermentation proceeds before iodoacetate inhibition, the greater the differential effect on fermentation and respiration, more ethanol having accumulated. If the yeast is washed immediately be-
before the iodoacetate is added, respiration is inhibited sooner and much more completely.

(c) Protection of 3-PGDH by 3-phosphoglyceraldehyde. The steady-state level of 3-phosphoglyceraldehyde may be higher under aerobic conditions than under anaerobic conditions, as shown in yeast by Holzer and Holzer (1953). Indeed, they found some protection aerobically. It is unlikely that this is a major factor in most cases.

(d) Alteration of the SH/SS ratio. Inasmuch as iodoacetate reacts only with SH groups and not with disulfide groups, Turner (1937) postulated that the SH/SS ratio may be lower aerobically than anaerobically, a greater fraction of the SH groups being unavailable for reaction, and showed that even small amounts of oxygen reduce the rate of inhibition of fermentation.

(e) Deviation of pyruvate metabolism. The pyruvate formed from the EM pathway normally may be oxidized through the cycle or reduced to lactate. Heald (1953) believed that iodoacetate might inhibit the latter more strongly because it also involves the utilization of NADH formed in the 3-PGDH reaction. As pyruvate formation is reduced, less goes to lactate but essentially as much into the cycle (see page 107). This applies, of course, to aerobic glycolysis only.

(f) Secondary interference with glucose phosphorylation. This has been sufficiently discussed (see page 74). Anaerobically the ATP level may be lower and fall more rapidly than aerobically, and thus the hexokinase reaction is depressed sooner under anaerobic conditions. Indeed, the inhibition of anaerobic glycolysis is a vicious circle, in that depression of 3-PGDH reduces the ATP, which slows glucose phosphorylation, further reducing the amount of 3-phosphoglyceraldehyde available for oxidation.

(g) Presence of alternate pathways for carbohydrate oxidation. Oxidative pathways for hexose utilization not involving an iodoacetate-sensitive step may account for part of the uninhibited respiration. These will be discussed in the following section.

Any one or a combination of these may account for the differential actions reported and the actual explanation in a particular instance must depend on experimental evidence. Most of the hypotheses have had very little or no experimental basis. On the basis of what evidence is available, one would conclude that explanations (a), (b), (f), and (g) are the most generally applicable. Those who believed fermentation and respiration to be entirely distinct (e.g., Cayrol and Genevois, 1931) and those who believed them inseparable (e.g., Ehrenfest, 1932) were in general both wrong, since in most cells respiration is to some degree dependent on the glycolytic breakdown of carbohydrate through the EM pathway, but is seldom either independent or completely dependent.
Action of Iodoacetate as Related to Alternate Pathways for Glucose Oxidation

The experimental evidence that oxidation of glucose in some tissues can proceed when the EM pathway is blocked by iodoacetate led some early investigators to conclude that a pathway existed alternate to the EM pathway and not necessarily involving 3-PGDH. We have seen above that there are several explanations for this phenomenon, and incomplete inhibition by iodoacetate cannot alone establish that an alternate pathway is operative. In fact, the cells in which the early evidence was obtained (mainly yeast and muscle) are now known to be rather deficient in these alternate pathways (e.g., yeast normally oxidizes approximately 3% of the glucose taken up via the pentose-P pathway). However, since it is now demonstrated beyond doubt that many cells possess an active pentose-P pathway, we must consider its role in the effects of iodoacetate on carbohydrate oxidation.

Scheme 2 shows some of the carbohydrate pathways. The relationships of the direct oxidation of glucose, the pentose-P pathway, and the Entner-Doudoroff pathway to the EM pathway are such that both CO₂ formation and O₂ uptake can occur in the presence of a block at the 3-PGDH step. The direct oxidation of glucose and the Entner-Doudoroff sequence are probably of importance only in certain cells, mostly microorganisms; e.g., the latter pathway is very important in the pseudomonads but perhaps not elsewhere. However, Ramachandran and Gottlieb (1963) have recently found that Caldariomyces fumago possesses not only the EM and pentose-P pathways but also the Entner-Doudoroff pathway and an active glucose oxidase of the notatin type. Iodoacetate at 1 mM inhibits the glucose respiration 50% in whole cells but only 9% in extracts; indeed, the cellular O₂ uptake is reduced to that of the extract, so that apparently the glucose oxidation in the extract is mediated through iodoacetate-resistant pathways. Glucose dehydrogenase occurs in liver but is usually not very active in other tissues, while glucose oxidases are confined to bacteria, fungi, and algae. We shall thus be particularly concerned with the pentose-P pathway, which is apparently more widespread than any other route alternate to the EM pathway. The operation of the pentose-P cycle leads to the formation of NADPH, CO₂, and 3-phosphoglyceraldehyde. The NADPH can reduce a variety of substances (directly or through transhydrogenation with NAD) or be oxidized by O₂ through electron transport systems. It is thus evident that, in cells in which the pentose-P pathway is active, respiration and aerobic CO₂ formation could occur in the presence of iodoacetate, providing iodoacetate does not block this pathway. Table 1-19 presents the available data on the inhibition of enzymes involved in the pentose-P pathway and certain other pathways of pentose metabolism. It is seen that none of these enzymes is markedly inhibited by iodoacetate and at concentrations of 1 mM and below there is likely to be very little if any effect on the pentose-P pathway, so that essentially a complete block of the EM pathway could
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Iodoacetate (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinose dehydrogenase</td>
<td><em>Pseudomonas saccharophila</em></td>
<td>10</td>
<td>0</td>
<td>Doudoroff <em>et al.</em> (1958)</td>
</tr>
<tr>
<td>Deoxyribose-5-P aldolase</td>
<td><em>Escherichia coli</em></td>
<td>0.1</td>
<td>0</td>
<td>Racker (1955)</td>
</tr>
<tr>
<td>Fructose-6-P phosphoketolase</td>
<td><em>Acetobacter xylinum</em></td>
<td>6</td>
<td>0</td>
<td>Schramm <em>et al.</em> (1958)</td>
</tr>
<tr>
<td>Galactose dehydrogenase</td>
<td><em>Pseudomonas saccharophila</em></td>
<td>10</td>
<td>0</td>
<td>Doudoroff <em>et al.</em> (1958)</td>
</tr>
<tr>
<td>Gluconate dehydrogenase</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>1</td>
<td>0</td>
<td>Ramakrishnan and Campbell (1955)</td>
</tr>
<tr>
<td>Gluconate kinase</td>
<td>Pig kidney</td>
<td>1</td>
<td>0</td>
<td>Leder (1957)</td>
</tr>
<tr>
<td>Glucose dehydrogenase</td>
<td><em>Aspergillus</em> sp.</td>
<td>2.2</td>
<td>0</td>
<td>Müller (1931)</td>
</tr>
<tr>
<td></td>
<td><em>Candida reukaufii</em></td>
<td>1.7</td>
<td>0</td>
<td>Bruchmann (1953)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8.6</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>43</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Streptomyces olivaceous</em></td>
<td>1</td>
<td>6</td>
<td>Maitra and Roy (1959)</td>
</tr>
<tr>
<td>Glucose-6-P dehydrogenase</td>
<td></td>
<td>40</td>
<td>33</td>
<td>Eichel and Wainio (1948)</td>
</tr>
<tr>
<td></td>
<td>Beef liver</td>
<td>1</td>
<td>0</td>
<td>Harrison (1931)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>5</td>
<td>Nakamura (1954)</td>
</tr>
<tr>
<td></td>
<td>Pea leaves</td>
<td>—a</td>
<td>0</td>
<td>Gibbs (1952)</td>
</tr>
<tr>
<td></td>
<td>Human erythrocytes</td>
<td>1</td>
<td>0</td>
<td>Kirkman (1962)</td>
</tr>
<tr>
<td></td>
<td>Rat liver</td>
<td>2.5a</td>
<td>12</td>
<td>Glock and McLean (1953)</td>
</tr>
<tr>
<td></td>
<td>Horse liver</td>
<td>10a</td>
<td>&lt;20</td>
<td>Dickens and Glock (1951)</td>
</tr>
<tr>
<td>2-Keto-3-deoxy-&lt;nobr&gt;d-araboh&lt;/nobr&gt;-leptonate-7-P synthetase</td>
<td><em>Escherichia coli</em></td>
<td>—</td>
<td>0</td>
<td>Srinivasan and Spriño (1959)</td>
</tr>
<tr>
<td>Enzyme/Dehydrogenase</td>
<td>Organism</td>
<td>K (M)</td>
<td>V (M)</td>
<td>References</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>---------------------------------</td>
<td>-------</td>
<td>-------</td>
<td>------------------------------------------------</td>
</tr>
<tr>
<td>Mannitol-1-P dehydrogenase</td>
<td><em>Escherichia coli</em></td>
<td>5</td>
<td>0</td>
<td>Wolff and Kaplan (1956)</td>
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<tr>
<td>Phosphoarabinoinosomerase</td>
<td><em>Propionibacterium pentosaceum</em></td>
<td>2.5</td>
<td>0</td>
<td>Volk (1960)</td>
</tr>
<tr>
<td>6-Phosphogluconate dehydrase</td>
<td><em>Pseudomonas fluororescens</em></td>
<td>1</td>
<td>11</td>
<td>Kovachevich and Wood (1955)</td>
</tr>
<tr>
<td>6-Phosphogluconate dehydrogenase</td>
<td><em>Leuconostoc mesenteroides</em></td>
<td>2</td>
<td>20</td>
<td>DeMoss and Gibbs (1955)</td>
</tr>
<tr>
<td></td>
<td><em>Streptomyces olivaceus</em></td>
<td>1</td>
<td>8</td>
<td>Maitra and Roy (1959)</td>
</tr>
<tr>
<td></td>
<td>Rat liver</td>
<td>2.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16</td>
<td>Glock and McLean (1953)</td>
</tr>
<tr>
<td></td>
<td>Horse liver</td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;20</td>
<td>Dickens and Glock (1951)</td>
</tr>
<tr>
<td>Phosphoribomutase</td>
<td>Beef uterus</td>
<td>0.6</td>
<td>0</td>
<td>Guarino and Sable (1956)</td>
</tr>
<tr>
<td>Ribose-P isomerase</td>
<td><em>Echinococcus granulosus</em></td>
<td>1</td>
<td>9</td>
<td>Agosin and Aravena (1960)</td>
</tr>
<tr>
<td></td>
<td>Rabbit muscle</td>
<td>50</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Erythrocytes</td>
<td>10</td>
<td>0</td>
<td>Dickens and Williamson (1956)</td>
</tr>
<tr>
<td></td>
<td>Human erythrocytes</td>
<td>10</td>
<td>0</td>
<td>Bruns &lt;i&gt;et al.&lt;/i&gt; (1958)</td>
</tr>
<tr>
<td></td>
<td>Sedoheptulose kinase</td>
<td>2</td>
<td>35</td>
<td>Urvetzky and Tsuboi (1963)</td>
</tr>
<tr>
<td></td>
<td><em>Bacillus sp.</em></td>
<td>10</td>
<td>60</td>
<td>Ebata &lt;i&gt;et al.&lt;/i&gt; (1955)</td>
</tr>
<tr>
<td>Transketolase</td>
<td>Yeast</td>
<td>—</td>
<td>0</td>
<td>Datta and Racker (1961)</td>
</tr>
<tr>
<td></td>
<td>Pig liver</td>
<td>5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20</td>
<td>Simpson (1960)</td>
</tr>
<tr>
<td>Xylose isomerase</td>
<td><em>Pseudomonas hydrophila</em></td>
<td>2</td>
<td>&lt;5</td>
<td>Hochster (1955)</td>
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<td></td>
<td></td>
<td>15</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Xylose reductase</td>
<td><em>Penicillium chrysogenum</em></td>
<td>10</td>
<td>0</td>
<td>Chiang and Knight (1959)</td>
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<tr>
<td>Xylulose kinase</td>
<td><em>Aerobacter aerogenes</em></td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>Bhuyan and Simpson (1962)</td>
</tr>
<tr>
<td></td>
<td>Calf liver</td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>Xylulose reductase</td>
<td><em>Saccharomyces rouxii</em></td>
<td>0.1</td>
<td>0</td>
<td>Hickman and Ashwell (1958)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>100</td>
<td>Blakley and Spence (1962)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Iodoacetamide.
Scheme 2. Carbohydrate pathways. (A), EM pathway; (B), pentose-P cycle; (C), direct glucose oxidation; (D), Entner-Doudoroff pathway.
occur without influencing this alternate route for glucose oxidation. There is 
also good evidence, as we shall see, that the pentose-P cycle can operate 
well in iodoacetate-treated cells.

Following the demonstration by Warburg and his co-workers in 1935 that 
a yeast extract can oxidize glucose-6-P to gluconate-6-P and then metab-
olize this product, Lipmann (1936) showed that bromoacetate at a cen-
truction which inhibits fermentation completely has little effect on this 
pathway. The oxygen uptake from ribose-5-P is not significantly inhibited 
in extracts of horse liver (Dickens and Glock, 1951), and the utilization of 
ribose-5-P by Lactobacillus brevis is slightly stimulated by iodoacetate (Eltz 
and Vandemark, 1960). The oxidation of ribose-5-P by Streptomyces is not 
inhibited by even 10 mM iodoacetate when NADP-dependent systems are 
used (Cochrane et al., 1953; Cochrane and Hawley, 1956), but in other cases 
the oxidation pathway may be through 3-PGDH, as in Pseudomonas hydro-
phila (Stone and Hochster, 1956), so that appreciable inhibition is exerted. 
Ordinarily it may be a mixed type of oxidation so that inhibition is partial, 
as in Streptomyces aureofaciens, where 2 mM iodoacetate inhibits 27%, the 
amount of sedoheptulose formed remaining constant but pyruvate forma-
tion being completely blocked (Sang-ch’ung and Yung-p’u, 1960). The evi-
dence from fragmentary studies on the oxidation of gluconate-6-P and 
pentose-P thus supports the results on isolated enzymes, and indicates an 
insensitivity of the pentose-P pathway to iodoacetate.

We shall now turn to data obtained by the recovery of C\textsuperscript{14}O\textsubscript{2} from glu-
cose-1-C\textsuperscript{14} and glucose-6-C\textsuperscript{14}, this providing information on the relative im-
portance of the two pathways and the effects of iodoacetate on the pattern 
of glucose oxidation. The results in Table 1-20 illustrate the variable re-
sponses observed, which is not unexpected since the patterns of glucose 
oxidation in different cells are not uniform. Exclusive metabolism through 
the EM pathway should lead to a C-1/C-6 ratio of 1; values greater than 1 
are generally considered to indicate the operation of the pentose-P path-
way. The incubation times are quite important since it is a matter of the 
relative rates at which C\textsuperscript{14}O\textsubscript{2} is formed from labeled glucose. For example, 
the increased glucose utilization and respiration associated with initiation 
of phagocytosis in leucocytes are accompanied by an increase in the con-
tribution from the pentose-P pathway, as shown by the rise in the C-1/C-6 
ratio (Table 1-20). If iodoacetate blocks the EM pathway at 3-PGDH spe-
cifically, one might expect the EM pathway contribution to be reduced, 
the formation of C\textsuperscript{14}O\textsubscript{2} from glucose-6-C\textsuperscript{14} inhibited more than from glucose-
1-C\textsuperscript{14}, and an increase in the C-1/C-6 ratio. Actually this is not commonly 
observed; except for the preparation of beef heart mitochondria and super-
nate in the presence of NADP or NADPH, where the formation of C\textsuperscript{14}O\textsubscript{2} 
from glucose-6-C\textsuperscript{14} is blocked completely, the C-1/C-6 ratio tends either to 
remain fairly constant or to decrease.
### Table 1-20

**Effects of iodoacetate on the Recovery of C\textsuperscript{14}O\textsubscript{2} from Labeled Glucose**

<table>
<thead>
<tr>
<th>Organism and tissue</th>
<th>Iodoacetate (mM)</th>
<th>% Change in C\textsuperscript{14}O\textsubscript{2} from:</th>
<th>C-1/C-6 Ratio</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Glucose-1-C\textsuperscript{14}</td>
<td>Glucose-6-C\textsuperscript{14}</td>
<td>Control</td>
</tr>
<tr>
<td><em>Chlorella pyrenoidosa</em></td>
<td>0.01</td>
<td>—</td>
<td>—</td>
<td>2.06</td>
</tr>
<tr>
<td></td>
<td>0.03</td>
<td>—</td>
<td>—</td>
<td>2.29</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>—</td>
<td>—</td>
<td>2.13</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>—</td>
<td>—</td>
<td>2.05</td>
</tr>
<tr>
<td>Lobster hepatopancreas</td>
<td>0.2</td>
<td>−78</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Calf thymus nuclei</td>
<td>1</td>
<td>—</td>
<td>−75</td>
<td>—</td>
</tr>
<tr>
<td>Guinea pig leucocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting</td>
<td>0.1</td>
<td>−49</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>−64</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Phagocytosing</td>
<td>0.1</td>
<td>−57</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>−63</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Resting</td>
<td>0.1</td>
<td>−29</td>
<td>+20</td>
<td>4.6</td>
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<td>Phagocytosing</td>
<td>0.1</td>
<td>−86</td>
<td>−31</td>
<td>22.7</td>
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<td>Rat mammary gland</td>
<td>0.1</td>
<td>−66</td>
<td>+38</td>
<td>21</td>
</tr>
<tr>
<td>Beef heart mitochondria</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>With NAD</td>
<td>0.17</td>
<td>−56</td>
<td>−39</td>
<td>1.36</td>
</tr>
<tr>
<td>With NADH</td>
<td>0.17</td>
<td>−61</td>
<td>−60</td>
<td>0.85</td>
</tr>
<tr>
<td>With NADP</td>
<td>0.17</td>
<td>−47</td>
<td>−100</td>
<td>2.34</td>
</tr>
<tr>
<td>With NADPH</td>
<td>0.17</td>
<td>−31</td>
<td>−100</td>
<td>1.44</td>
</tr>
<tr>
<td>Guinea pig brain slices</td>
<td>0.4</td>
<td>−62</td>
<td>−63</td>
<td>1.10</td>
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<tr>
<td>Walker rat carcinoma</td>
<td>0.1</td>
<td>+22</td>
<td>+7</td>
<td>2.80</td>
</tr>
<tr>
<td>Ehrlich ascites carcinoma cells</td>
<td>0.07</td>
<td>+2</td>
<td>+10</td>
<td>1.81</td>
</tr>
<tr>
<td></td>
<td>0.17</td>
<td>−49</td>
<td>−16</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0.35</td>
<td>−58</td>
<td>−33</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>−80</td>
<td>−51</td>
<td>—</td>
</tr>
</tbody>
</table>
The determination and interpretation of C-1/C-6 ratios are complicated by many factors. The operation of the pentose-P pathway may be limited by the rate of reoxidation of NADPH. In many cells there is apparently a deficiency of NADPH oxidases and similar systems. In ascites cells the principal hydrogen acceptor for NADPH is pyruvate (Wenner, 1959). Thus pyruvate will overcome the effects of iodoacetate (see accompanying tabulation). When iodoacetate prevents the formation of pyruvate, NADPH cannot be oxidized readily, and the pentose-P pathway slows. Thus iodoacetate can indirectly depress the pentose-P pathway. McLean (1962) found that phenazine methosulfate can counteract the inhibition of C\textsuperscript{14}O\textsubscript{2} formed from glucose-1-C\textsuperscript{14} by iodoacetate in rat mammary gland, and postulated that the phenazine methosulfate acts as an alternative electron acceptor, thereby restoring the activity of the pentose-P pathway. She also obtained evidence that the pentose-P pathway and fatty acid synthesis are tightly geared, and that iodoacetate depresses the pentose-P pathway, not by acting on it directly but by interfering with the enzymes of the malonyl-CoA pathway. Such interrelationships may at least partly explain the rather marked inhibitions of the pentose-P pathway activity by iodoacetate. If a large fraction of the pyruvate formed from the EM pathway is not oxidized through the cycle but goes into various syntheses, the C\textsuperscript{14}O\textsubscript{2} from glucose-6-C\textsuperscript{14} may be quite low (the 6-C\textsuperscript{14} ends up as the methyl group on pyruvate so that it can be released as C\textsuperscript{14}O\textsubscript{2} only after recycling). If iodoacetate affects in any way the distribution of pyruvate, it will alter the C-1/C-6 ratio; e.g., if iodoacetate increases the relative amount of pyruvate going into the cycle, the formation of C\textsuperscript{14}O\textsubscript{2} from glucose-6-C\textsuperscript{14} will be inhibited less than the EM pathway. These considerations make the interpretation of such experiments very difficult. An increase in the formation of C\textsuperscript{14}O\textsubscript{2} from glucose-6-C\textsuperscript{14} by iodoacetate would not be expected, yet occurs in the mammary gland and other tissues; is this due to the fact that fatty acid synthesis is markedly inhibited and more pyruvate is diverted into the cycle? An increase in C\textsuperscript{14}O\textsubscript{2} from glucose-1-C\textsuperscript{14} by iodoacetate can also occur (as in Walker rat carcinoma) and is perhaps related to the accumulation of glucose-6-P. Ferricyanide, which acts earlier in the EM pathway

<table>
<thead>
<tr>
<th>Pyruvate (mM)</th>
<th>Iodoacetate (mM)</th>
<th>C-1/C-6 Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>—</td>
<td>5.29</td>
</tr>
<tr>
<td>10</td>
<td>—</td>
<td>10.5</td>
</tr>
<tr>
<td>—</td>
<td>2</td>
<td>2.89</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>5.39</td>
</tr>
</tbody>
</table>
than iodoacetate, stimulates the pentose-P pathway strongly and was postulated by Birkenhāger (1959) possibly to inhibit phosphohexokinase, this bringing about a greater accumulation of glucose-6-P. However, there is very little evidence that iodoacetate can in general increase the utilization of glucose through the pentose-P pathway.

The pentose-P pathway is present in most tumor tissues and may actually be slightly more active than in normal tissues (Aisenberg, 1961, p. 88). In addition to the two carcinomas in Table 1-20, several tumors have been studied less intensively. Villavicencio and Barron (1957) found iodoacetate to inhibit aerobic glucose utilization less readily in lymphosarcoma than in lymphatic cells, and concluded that the pentose-P cycle is more important in the tumor, while Racker (1956) attributed the failure of iodoacetate to inhibit glucose respiration in ascites cells to some alternate pathway for glucose oxidation. Iodoacetate at concentrations completely blocking glycolysis inhibits 50-75% the formation of C14O2 from glucose-u-C14 and glucose-6-C14 in a variety of tumors, so that an alternate pathway is likely, although one cannot be certain that aerobically the EM pathway is fully blocked (van Vals et al., 1956; van Vals and Emmelot, 1957).

Another type of evidence for the operation of alternate pathways is the differential effects sometimes observed on the oxidation of glucose, hexose monophosphates, and hexose diphosphate. Some of the results and the difficulties involved have been described (page 125) and here we shall note only briefly some data obtained aerobically. If the pentose-P pathway is operative, the oxidation of fructose-1,6-diP may be inhibited more readily than that of glucose or glucose-6-P. This has been found in Sarcina lutea (Barron and Friedemann, 1941), tobacco leaves (Clayton, 1959), sea urchin eggs (Lindberg and Ernstn, 1948), and adrenal tissue (Kelly et al., 1955). In other instances the oxidation of hexose monophosphates is inhibited by iodoacetate completely, as in grasshopper embryos (Bodine and West, 1953) and pea seeds (Hatch and Turner, 1958), so that a normal EM pathway with no pentose-P cycle is indicated. The oxidation of fructose-1,6-diP in Streptomyces coelicolor is inhibited only 22% by 2 mM iodoacetate, whereas 3-PGDH is blocked completely by 1 mM (Cochrane, 1955). In this case, fructose-1,6-diP may be hydrolyzed to the monophosphate which can proceed through the pentose-P pathway. However, another possibility is the phosphorolytic cleavage of fructose-6-P by a phosphoketolase, such as was found in Actobacter xylinum by Schramm et al. (1958), acetyl-P being formed along with erythrose-4-P,

\[
\text{Fructose-6-P} + P_i \rightarrow \text{acetyl-P} + \text{erythrose-4-P} + H_2O
\]

which can be metabolized to acetyl-P or acetate.
Effect on Respiratory Quotient (R.Q.)

The change of R.Q. brought about by iodoacetate might provide some information as to the metabolic disturbance produced if care is taken in the interpretation of such changes in complex systems. If iodoacetate blocks the EM pathway specifically and if the cells are oxidizing carbohydrates and other substrates, one would expect a fall in the R.Q., since the carbohydrate fraction of the metabolism should be reduced. However, if an active pentose-P cycle is present, an increase in the R.Q. is possible, especially if some of the NADPH is oxidized by any pyruvate present. Actually, the change in R.Q. induced by iodoacetate is often quite small (Table 1-21); in perhaps ten of the tissues in the table the change is insignificant (in some of them the iodoacetate affects none of the tissue's metabolic activities, e.g., respiration, so that insufficient concentration due to poor penetration may be the reason for the lack of effect on the R.Q.). In many cells there is a definite drop in the R.Q., especially when glucose is provided in the medium; if the metabolism is endogenous, there may be little carbohydrate being oxidized so that the effect on the R.Q. cannot be predicted. Corn root is the only tissue showing a really marked increase in the R.Q. due to iodoacetate when glucose is present, although cat and dog heart and cat brain show slight increases. Although accurate figures are not available, it is clear that the R.Q. is also decreased in rhubarb leaves (Morrison, 1950), several yeasts (Spiegelman and Nozawa, 1945), and dog heart (Burns and Cruickshank, 1937). An important factor often neglected is the presence of various intermediates or products of carbohydrate metabolism, so that, after iodoacetate is added, oxidation of these substances can continue for a time. For example, ethanol would be completely oxidized with an R.Q. of 0.67 and might contribute to reducing the over-all R.Q., while lactate would be completely oxidized with an R.Q. of 1.0, both lactate and pyruvate giving R.Q.'s higher than 1.0 if the oxidation is incomplete, thereby contributing to a rise in the R.Q. By themselves, R.Q. studies do not tell us a great deal about the action of iodoacetate, but in conjunction with other data may have some value in elucidating the complex metabolic changes which occur.

Effects on the General Patterns of Glucose Oxidation and Metabolism

There now remain to be discussed some of the effects on carbohydrate metabolism not covered in the previous sections to round out our picture of the alterations produced by iodoacetate. One problem is the synthesis of di- and polysaccharides from glucose. If glucose oxidation is impaired by iodoacetate, will relatively more go into synthetic pathways? The answer seems to be definitely in the negative. The following syntheses are readily depressed by iodoacetate: glycogen in yeast (Brücke, 1933), in rat liver slices (Bach, 1939), and in rat diaphragm (Haft and Mirsky, 1952);
<table>
<thead>
<tr>
<th>Organism and tissue</th>
<th>Substrate</th>
<th>Iodoacetate (mM)</th>
<th>R. Q.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td>Inhibited</td>
</tr>
<tr>
<td>Yeast suspension</td>
<td>Glucose</td>
<td>1.1</td>
<td>1.38</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>1.0</td>
<td>1.33</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>0.2</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Yeast extract + ATP</td>
<td>None</td>
<td>1.0</td>
<td>0.96</td>
<td>1.51</td>
</tr>
<tr>
<td>Carrot root slices</td>
<td>Glucose</td>
<td>0.054</td>
<td>1.08</td>
<td>1.00</td>
</tr>
<tr>
<td>Barley roots</td>
<td>None</td>
<td>0.01</td>
<td>0.97</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05</td>
<td>0.97</td>
<td>0.89</td>
</tr>
<tr>
<td>Pea stems + auxin</td>
<td>None</td>
<td>0.6</td>
<td>0.98</td>
<td>1.07</td>
</tr>
<tr>
<td>Corn root tips</td>
<td>Glucose</td>
<td>1.0</td>
<td>1.2</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td></td>
<td>1.2</td>
<td>5.5</td>
</tr>
<tr>
<td>Sea urchin spermatozoa</td>
<td>None</td>
<td>1.0</td>
<td>1.08</td>
<td>1.12</td>
</tr>
<tr>
<td>Grasshopper embryo</td>
<td>None</td>
<td>1.0</td>
<td>0.83</td>
<td>0.95</td>
</tr>
<tr>
<td>Tissue</td>
<td>Sugar</td>
<td>0.1–10</td>
<td>1.2</td>
<td>1.3–1.5</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------</td>
<td>--------</td>
<td>-----</td>
<td>---------</td>
</tr>
<tr>
<td>Codling moth larval muscle</td>
<td>None</td>
<td>0.1–10</td>
<td>1.2</td>
<td>1.3–1.5</td>
</tr>
<tr>
<td>Frog eggs</td>
<td>None</td>
<td>3.3</td>
<td>1.0</td>
<td>0.83</td>
</tr>
<tr>
<td>Frog muscle</td>
<td>Glucose</td>
<td>0.25</td>
<td>0.91</td>
<td>0.77</td>
</tr>
<tr>
<td>Frog muscle</td>
<td>Glucose</td>
<td>0.32</td>
<td>0.91</td>
<td>0.91</td>
</tr>
<tr>
<td>Frog heart</td>
<td>Glucose</td>
<td>12</td>
<td>0.87</td>
<td>0.78</td>
</tr>
<tr>
<td>Chick embryo erythrocytes</td>
<td>Glucose</td>
<td>0.5</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Chick embryo midbrain</td>
<td>Glucose</td>
<td>0.04</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Ox retina</td>
<td>Glucose</td>
<td>0.1</td>
<td>0.98</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>0.98</td>
<td>0.89</td>
</tr>
<tr>
<td>Dog skeletal muscle</td>
<td>None</td>
<td>0.54</td>
<td>0.87</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>0.54</td>
<td>1.03</td>
<td>0.97</td>
</tr>
<tr>
<td>Dog heart</td>
<td>None</td>
<td>0.072</td>
<td>0.83</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>0.072</td>
<td>0.91</td>
<td>0.95</td>
</tr>
<tr>
<td>Cat intestinal muscle</td>
<td>Glucose</td>
<td>0.54</td>
<td>1.00</td>
<td>0.98</td>
</tr>
<tr>
<td>Cat heart</td>
<td>Glucose</td>
<td>0.054</td>
<td>0.86</td>
<td>0.92</td>
</tr>
<tr>
<td>Cat brain</td>
<td>None</td>
<td>0.054</td>
<td>1.01</td>
<td>1.03</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>0.054</td>
<td>0.99</td>
<td>1.04</td>
</tr>
</tbody>
</table>
lactose in mammary gland (Grant, 1936); sucrose in pea seeds (Turner, 1957); and cellulose in *Acetobacter xylinum* (Schramm et al., 1957). I know of no case in which carbohydrate synthesis is increased by iodoacetate; this is perhaps related, in part, to the fall in ATP associated with the action of iodoacetate.

What effect will iodoacetate have on the total uptake or utilization of glucose? Since the factors involved here are many and complex it is difficult to predict the over-all result. Most cells probably do not have high intracellular levels of free glucose and the level would be expected to rise after addition of iodoacetate, particularly in those cells not possessing an active pentose-P pathway. This occurs very markedly in rabbit erythrocytes, the glucose concentration rising from 16 mg% to 392 mg% in 5 hr in the presence of 1 mM iodoacetate (Laris, 1958). We have noted that glucose accumulation occurs in yeast treated with iodoacetate, concentrations as high as 40–55 mM in the cell water being reached (Cirillo, 1962). These cells were incubated with external glucose, but if the tissues are bathed in glucose-free media the effect of iodoacetate is often to accelerate the loss of glucose from the cells. For example, in pea stems the initial glucose is 1.12%, after incubation for 24 hr with auxin is 0.86%, and in the presence of auxin and 0.6 mM iodoacetate is 0.61% (Christiansen et al., 1949); in perfused frog liver, iodoacetate at 0.3–2 mM doubles the glucose output, this presumably being mainly from glycologen breakdown and indicating less utilization (Craig, 1959). The uptake and oxidation of glucose in adipose tissue are accelerated by insulin. Iodoacetate concentrations having no effect on glucose oxidation directly inhibit the stimulation produced by insulin (Mirsky and Perisutti, 1962). The binding of insulin to the tissue is not prevented, so one must assume that an iodoacetate-sensitive uptake mechanism is initiated by insulin. Human leucocytes accumulate galactose to intracellular concentrations 3–6 times the external, and convert the galactose to galactose-1-P, UDP-galactose, and other products. Iodoacetate at 1 mM increases the total radioactivity and the labeled galactose and galactose-1-P in the cells, when the leucocytes are incubated with galactose-C\textsuperscript{14} (Kalant and Schucher, 1963). This might be explained by a block of the metabolism of galactose-1-P or, more tenously, by assuming that glycolysis inhibits the transport of galactose so that depressed glycolysis would accelerate galactose uptake. Hexose uptake is a complex phenomenon controlled by many factors and the effects of iodoacetate have not yet been clearly delineated.

We shall now consider direct experiments on glucose uptake; some results are given in Table 1-22. It is seen that glucose uptake is almost uniformly inhibited, although not to the same extent as glycolysis in most cases. The stimulation of glucose uptake by yeast at 0.1 mM iodoacetate is interesting and is accompanied by a comparable stimulation of respira-
Table 1-22

Effects of Iodoacetate on Over-All Glucose Uptake or Utilization

<table>
<thead>
<tr>
<th>Organism and tissue</th>
<th>Iodoacetate (mM)</th>
<th>% Inhibition glucose utilization</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Brevibacterium flavum</em></td>
<td>1</td>
<td>100</td>
<td>Shiio <em>et al.</em> (1961)</td>
</tr>
<tr>
<td><em>Propionibacterium freundenreichii</em></td>
<td>0.05</td>
<td>24</td>
<td>Field and Lichstein (1958)</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>Yeast</td>
<td>0.1</td>
<td>Stim 17</td>
<td>Stickland (1956 b)</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Beef kidney culture cells</td>
<td>0.1</td>
<td>90</td>
<td>Polatnick and Bachrach (1960)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Rat diaphragm</td>
<td>4</td>
<td>Stim 20</td>
<td>Haft and Mirsky (1952)</td>
</tr>
<tr>
<td>Mammary gland</td>
<td>20</td>
<td>100</td>
<td>Grant (1936)</td>
</tr>
<tr>
<td>Guinea pig brain slices</td>
<td>0.03</td>
<td>19</td>
<td>Takagaki <em>et al.</em> (1958)</td>
</tr>
<tr>
<td>Beef retina</td>
<td>Glucose = 5 mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>6</td>
<td>Hopkinson and Kerly (1959)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Glucose = 15 mM</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>36</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>HeLa cells</td>
<td>0.3</td>
<td>85</td>
<td>Wu (1959)</td>
</tr>
<tr>
<td>Mouse mammary carcinoma</td>
<td>0.001</td>
<td>0</td>
<td>Young and Taylor (1953)</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Ehrlich ascites carcinoma cells</td>
<td>0.035</td>
<td>41</td>
<td>Maizels <em>et al.</em> (1958)</td>
</tr>
<tr>
<td></td>
<td>0.07</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>14</td>
<td>Kvakme (1958 b)</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>82</td>
<td>Wu and Racker (1959)</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>61</td>
<td>Seelich and Lethnansky (1960)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>69</td>
<td>Laws and Stickland (1962)</td>
</tr>
</tbody>
</table>

At the same concentration, anaerobic glucose utilization is inhibited 33% (Stickland, 1956 b). The Pasteur quotient \( Q_{gluc}^{O_2} / Q_{gluc}^{N_2} \) is thus increased markedly by iodoacetate, as much as 600% at 1 mM, which does not necessarily mean that the Pasteur reaction is inhibited since respiration...
is also depressed. The patterns of aerobic glucose metabolism in yeast as affected by iodoacetate are well seen in the accompanying tabulation. The decrease in total glucose utilized is due mainly to the reduction in the fraction anabolized, which is rather surprising and hardly explainable entirely on the basis of an action exclusively on 3-PCDH. In brain slices the situation is somewhat different, in that 0.03 mM iodoacetate inhibits glucose utilization 19%, glucose oxidized (calculated) 28%, lactate formed 9%, and respiration 4% (Takagaki et al., 1958). In ox retina the reduction in glucose utilization by iodoacetate is also probably due mainly to the inhibition of the EM pathway and the fraction of glucose being oxidized or going to lactate (Hopkinson and Kerly, 1959). Since in some cells (e.g. ascites cells) glucose inhibits the respiration (Crabtree effect), the effect of iodoacetate is particularly interesting. Would iodoacetate stimulate the respiration from its glucose-depressed level by blocking its metabolism? Ibsen et al. (1958) state that it does not, although their results indicate that slight stimulation may possibly occur with low iodoacetate concentrations. However, iodoacetate reduces the Crabtree effect, as also reported by Laws and Stickland (1962). Quite other results were obtained by Wu and Racker (1959), who found the Crabtree effect to be even greater in the presence of iodoacetate (see accompanying tabulation), which means that iodoacetate

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Iodoacetate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.25 mM</td>
</tr>
<tr>
<td>Total glucose utilized</td>
<td>0.76</td>
<td>0.68</td>
</tr>
<tr>
<td>Glucose catabolized</td>
<td>0.24</td>
<td>0.26</td>
</tr>
<tr>
<td>Oxidized</td>
<td>0.24</td>
<td>0.22</td>
</tr>
<tr>
<td>Fermented</td>
<td>0.00</td>
<td>0.04</td>
</tr>
<tr>
<td>Glucose anabolized</td>
<td>0.52</td>
<td>0.42</td>
</tr>
<tr>
<td>Into polysaccharide</td>
<td>0.34</td>
<td>0.26</td>
</tr>
<tr>
<td>Unaccounted for</td>
<td>0.18</td>
<td>0.16</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Additions</th>
<th>O₂ Uptake</th>
<th>Crabtree effect</th>
<th>Cellular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mM)</td>
<td>Iodoacetate (mM)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>---</td>
<td>31</td>
<td>35%</td>
</tr>
<tr>
<td>---</td>
<td>0.1</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>0.1</td>
<td>23</td>
<td>50%</td>
</tr>
</tbody>
</table>
inhibits glucose respiration more than endogenous respiration. If the Crabtree effect is due to depletion of $P_i$ and adenine nucleotides, iodoacetate does this more effectively when glucose is present, the $\text{AMP} : \text{ADP} : \text{ATP}$ ratio shifting from $0 : 0.7 : 1.64$ to $0.58 : 0.12 : 0.04$.

**Chronological Sequence of Events Brought About Aerobically by Iodoacetate**

The changes induced by iodoacetate under aerobic conditions are usually more complex than anaerobically (see page 84) because more pathways are often available and more substrates involved. The principal differences from the anaerobic situation will be outlined.

(A) Instead of all carbohydrate metabolism coming to a halt, there will be some continuation of respiration, $\text{CO}_2$ formation, glucose uptake, and perhaps even some formation of pyruvate if alternate pathways are available. The resistant respiration will be due mainly to substrates other than carbohydrate and the operation of the pentose-P cycle.

(B) It is possible that the accumulation of hexose phosphates will increase the pentose-P cycle activity if oxidation of NADPH is not limiting the rate.

(C) The production of high-energy bonds will slow, unless other substrates (e.g. fatty acids) are dominant, leading to transphosphorylation from creatine-P and eventual fall in ATP level. If glucose oxidation is the only or major metabolism, the rate of formation of ATP will be reduced proportionally to the inhibition of the EM pathway. However, there is less likelihood of a marked fall in ATP aerobically, so that a secondary effect on the hexokinase reactions is probably less important.

(D) Over-all phosphate metabolism may be very complex since there are so many compounds containing phosphate groups, and it is impossible to predict what will happen to the levels of any particular phosphate. One can generally say that the conservation equation:

$$\Delta A - P + \Delta B - P + \Delta C - P + \ldots + \Delta N - P + \Delta P_i = 0$$

where $A - P$, $B - P$, etc., refer to various phosphates, applies to the whole system, but not necessarily to the cells alone since phosphate may be taken up or lost by the cells. As in the anaerobic case, the changes in the nucleotides will depend strongly on the energy demand of the cells. Cell function will not so readily fail aerobically if iodoacetate acts selectively on the EM pathway, because of the other sources for ATP, but this will not necessarily hold if concentrations depressing pyruvate oxidation are used.
EFFECTS ON LIPID METABOLISM

Our knowledge of the effects of iodoacetate on lipid metabolism and most noncarbohydrate metabolism, is meager and gappy, and the problem is complicated by the interrelationships between glycolysis and almost all other metabolic pathways, so that even though the block is specific for 3-PGDH, quite possibly lipid metabolism will be altered secondarily by changes in the level of ATP or acetyl-CoA. In addition, one must always consider the direct action of iodoacetate on the fatty acid helix, transfers of two-carbon fragments, and the various biosynthetic pathways, although very few studies on these systems have been done. Table 1-23 presents a number of enzymes involved in lipid metabolism which are sensitive enough to warrant consideration even when iodoacetate is used at the low concentrations sufficient to block the EM pathway. The discussion will be divided into two parts: lipid catabolism and lipid synthesis.

Lipid Catabolism and Fatty Acid Oxidation

The oxidation of fatty acids is inhibited fairly potently by iodoacetate, as shown by Singer and Barron (1945) in suspensions of $E. \text{coli}$, 1 mM iodoacetate reducing the oxidation of stearate and olate around 45%, and confirmed by Waltman and Rittenberg (1954) in Serratia marcescens, where some inhibition was noted at 0.1 mM. The conversion of hexanoate and $\Delta^2$-hexenoate to acetoacetate in rat liver homogenates is also inhibited by iodoacetate, 1.3 mM depressing 31% and 60%, respectively (Witter et al., 1950). The site of this inhibition is unknown. Most of the helix enzymes have not been examined for sensitivity to iodoacetate; crotonase seems to be fairly resistant. One possible site is the initial activation of fatty acids, since Jencks and Lipmann (1957) found this system in pig liver to be inhibited 55–60% by 3 mM iodoacetate. In most of these test systems, ATP is added but, in the cell, iodoacetate may further depress this initial step by reducing the ATP available. In plants these oxidations may be less sensitive; at least the formation of $\text{C}^{14}\text{O}_2$ from palmitate-1-$\text{C}^{14}$ in extracts of peanut cotyledons is unaffected by 1 mM iodoacetamide (Castelfranco et al., 1955).

The interesting studies of Geyer and his co-workers (1950a, b) on fatty acid breakdown in rat liver slices provide evidence that perhaps a major portion of the iodoacetate inhibition is exerted on the helix. First, pyruvate does not counteract the effect of iodoacetate, which it should do if the site of action is the EM pathway and ATP is reduced (the rather high concentration of iodoacetate used, 5 mM, may have inhibited pyruvate oxidation, however). Second, the formation of labeled acetoacetate from labeled fatty acids is inhibited (see accompanying tabulation). The greater inhibition of $\text{C}^{14}\text{O}_2$ formation may reflect some effect on the cycle. Since 5 mM iodoacetate depresses the helix only 45%, in liver at least it is likely that concentrations
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Iodoacetate (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Acetacetate decarboxylase</td>
<td>Clostridium acetobutylicum</td>
<td>0.5</td>
<td>10</td>
<td>Seely (1955)</td>
</tr>
<tr>
<td>Acetyl-CoA carboxylase</td>
<td>Wheat germ</td>
<td>0.5</td>
<td>63</td>
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<td>0.5</td>
<td>67</td>
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<tr>
<td>Butyrate kinase</td>
<td>Clostridium butyricum</td>
<td>0</td>
<td>0</td>
<td>Twarog and Wolfe (1962)</td>
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<tr>
<td>Cholesterol esterase</td>
<td>Rat liver</td>
<td>0.5</td>
<td>1</td>
<td>Deykin and Goodman (1962)</td>
</tr>
<tr>
<td>Cholesterol esterase</td>
<td>Beef liver</td>
<td>4</td>
<td>2</td>
<td>Waikl and Mahler (1954)</td>
</tr>
<tr>
<td>Cholesterol esterase</td>
<td>Pig liver</td>
<td>3</td>
<td>50</td>
<td>Shapira and der Cauwille (1956)</td>
</tr>
<tr>
<td>Cholesterol esterase</td>
<td>Rat liver</td>
<td>53</td>
<td>50</td>
<td>Jencks and Lipmann (1957)</td>
</tr>
<tr>
<td>Cholesterol esterase</td>
<td>Rat adipose tissue</td>
<td>60</td>
<td>3</td>
<td>Lang and Kornfeld (1959)</td>
</tr>
<tr>
<td>Cholesterol esterase</td>
<td>Rat liver</td>
<td>10</td>
<td>0</td>
<td>Shapira and der Cauwille (1956)</td>
</tr>
<tr>
<td>Cholesterol esterase</td>
<td>Carrot</td>
<td>10</td>
<td>0</td>
<td>Nandi and Porter (1954)</td>
</tr>
<tr>
<td>Cholesterol esterase</td>
<td>Pig liver</td>
<td>10</td>
<td>0</td>
<td>Nandi and Porter (1954)</td>
</tr>
<tr>
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<td>Spinach</td>
<td>0.01</td>
<td>0</td>
<td>Holzer and Holdorf (1957)</td>
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<tr>
<td>Cholesterol esterase</td>
<td>Glycerate dehydrogenase</td>
<td>0.1</td>
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EFFECTS ON LIPID METABOLISM
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Iodoacetate (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerate kinase</td>
<td><em>Brassica campestris</em></td>
<td>1.8</td>
<td>66</td>
<td>Ozaki and Wetter (1969)</td>
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<td>Glycerol kinase</td>
<td><em>Candida mycoderma</em></td>
<td>0.5</td>
<td>50</td>
<td>Bergoeyer et al. (1961)</td>
</tr>
<tr>
<td>Glycerylphosphorylcholine diesterase</td>
<td>Rat liver</td>
<td>0.5</td>
<td>100</td>
<td>Dawson (1956a)</td>
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<td>Glycerylphosphorylcholine dehydratase</td>
<td>Yeast</td>
<td>2.5</td>
<td>0</td>
<td>Yamashita (1969)</td>
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<tr>
<td>Pig kidney</td>
<td>Pig heart</td>
<td>100</td>
<td>0</td>
<td>Robinson and Coon (1957)</td>
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<td>Pig heart</td>
<td>Pig heart</td>
<td>30</td>
<td>56</td>
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<td>Pig heart</td>
<td>5</td>
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<td>Beef heart</td>
<td>5</td>
<td>72</td>
<td>Sekuzu et al. (1963)</td>
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<td>Beef heart</td>
<td>2.5</td>
<td>0</td>
<td>Stern and Miller (1959)</td>
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<tr>
<td>Rat liver</td>
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<td>100</td>
<td>100</td>
<td>Stern and Miller (1959)</td>
</tr>
<tr>
<td>Rat liver</td>
<td>Rat liver</td>
<td>2.5</td>
<td>0</td>
<td>Stern and Miller (1959)</td>
</tr>
<tr>
<td>Wheat germ</td>
<td><em>Ophiidion elongatus</em> (lunged)</td>
<td>5</td>
<td>55</td>
<td>Singer (1948)</td>
</tr>
<tr>
<td>Pancreas</td>
<td><em>Singer and Barron</em> (1945)</td>
<td>1.25</td>
<td>21</td>
<td>Singer and Barron (1945)</td>
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<td>Lipase</td>
<td><em>Weinstein and Wynn</em> (1936)</td>
<td>1.35</td>
<td>32</td>
<td>Wills (1960)</td>
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<td>Enzyme</td>
<td>Organism</td>
<td>Inhibitor</td>
<td>IC50</td>
<td>Concentration</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>-----------------------------------</td>
<td>-----------</td>
<td>------</td>
<td>---------------</td>
</tr>
<tr>
<td>Malonyl-CoA—CO₂ exchange enzyme</td>
<td><em>Clostridium kluyveri</em></td>
<td>0.01</td>
<td>0</td>
<td>Vagelos and Alberts (1960)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>25</td>
<td></td>
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<tr>
<td>Malonyl-CoA transacylase</td>
<td><em>Escherichia coli</em></td>
<td>0.008</td>
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<td>Alberts <em>et al.</em> (1963)</td>
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<tr>
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<td>Brodie <em>et al.</em> (1963)</td>
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<td></td>
<td>5</td>
<td>35</td>
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<td></td>
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<td>β-Methylcrotonyl-CoA carboxylase</td>
<td><em>Achromobacter</em> sp.</td>
<td>1</td>
<td>0</td>
<td>Himes <em>et al.</em> (1963)</td>
</tr>
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<td></td>
<td></td>
<td>10</td>
<td>100</td>
<td></td>
</tr>
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<td>Methylmalonyl:oxalacetate transcarboxylase</td>
<td><em>Propionibacterium shermanii</em></td>
<td>0.1</td>
<td>13</td>
<td>Wood <em>et al.</em> (1963)</td>
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<td>Mevalonate dehydrogenase</td>
<td>Rat liver</td>
<td>0.1</td>
<td>50</td>
<td>Nakamura and Greenberg (1961)</td>
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<tr>
<td></td>
<td></td>
<td>1</td>
<td>100</td>
<td></td>
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<td>Mevalonate kinase</td>
<td><em>Cucurbita pepo</em> (pumpkin)</td>
<td>10</td>
<td>20</td>
<td>Loomis and Battale (1963)</td>
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<td>Phosphatidate phosphatase</td>
<td>Pig kidney</td>
<td>5</td>
<td>Stim 5</td>
<td>Coleman and Hübscher (1962)</td>
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<td>Phospholipase B</td>
<td>Rat liver</td>
<td>5</td>
<td>1</td>
<td>Dawson (1956 b)</td>
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<tr>
<td>Phosphomevalonate kinase</td>
<td>Pig liver</td>
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<td>9</td>
<td>Hellig and Popjak (1961)</td>
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<td>Propionyl-CoA carboxylase</td>
<td>Beef liver</td>
<td>0.1</td>
<td>0</td>
<td>Haleniz <em>et al.</em> (1962)</td>
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<td></td>
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<td>Stearate dehydrogenase</td>
<td>Rat liver</td>
<td>1</td>
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<td>Singer and Barron (1945)</td>
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<td>Δ⁴-Steroid dehydrogenase</td>
<td><em>Pseudomonas testosteroni</em></td>
<td>1</td>
<td>17</td>
<td>Levy and Talalay (1959)</td>
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<tr>
<td></td>
<td></td>
<td>1</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>Δ⁴-5α-Steroid dehydrogenase</td>
<td><em>Pseudomonas testosteroni</em></td>
<td>1</td>
<td>8</td>
<td>Levy and Talalay (1959)</td>
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<td></td>
<td></td>
<td>1</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Steroid hydroxylase</td>
<td>Beef adrenals</td>
<td>1</td>
<td>8</td>
<td>Ryan and Engel (1957)</td>
</tr>
</tbody>
</table>

* Iodoacetamide.
1. IODOACETATE AND IODOACETAMIDE

### Radioactivity

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Iodoacetate (5 mM)</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>A $\text{C}^{14}\text{O}_2$</td>
<td>9,830</td>
<td>1,890</td>
<td>− 81</td>
</tr>
<tr>
<td>B Acetoacetate-$\text{C}^{14}$O</td>
<td>4,200</td>
<td>2,340</td>
<td>− 44</td>
</tr>
<tr>
<td>C Acetoacetate-$\text{C}^{14}$O$^2$</td>
<td>7,770</td>
<td>4,300</td>
<td>− 45</td>
</tr>
<tr>
<td>D Total acetoacetate-$\text{C}^{14}$</td>
<td>11,970</td>
<td>6,640</td>
<td>− 45</td>
</tr>
<tr>
<td>A + D</td>
<td>21,800</td>
<td>8,530</td>
<td>− 61</td>
</tr>
<tr>
<td>A/D</td>
<td>0.82</td>
<td>0.28</td>
<td>− 66</td>
</tr>
<tr>
<td>B/C</td>
<td>0.54</td>
<td>0.54</td>
<td>0</td>
</tr>
</tbody>
</table>

* Radioactivity in counts/min/g dry wt.

below 0.5 mM will not produce much direct effect on fatty acid oxidation.

The oxidation of glycerol by bacteria is inhibited potently by iodoacetate − 63% by 0.1 mM and 86% by 0.5 mM (Mickelson and Shideman, 1947) — which is not surprising since the pathway postulated is

\[
\text{Glycerol} \rightarrow \alpha\text{-glycerol-P} \rightarrow 3\text{-P-glyceraldehyde} \rightarrow \text{P-glycerate}
\]

and involves glycerol kinase (which is sensitive, as seen in Table 1-23) and 3-PGDH. Inasmuch as the inhibition of the oxidation of $\alpha$-glycerol-P is just as strong, it is likely that the major site is 3-PGDH. It is surprising that G.J.E. Hunter (1953) found only 34–68% inhibition of glycerol oxidation by 10 mM iodoacetate in various mycobacteria. The oxidation of lysolecithin by avian *Mycobacterium tuberculosis* is reduced 19% by 0.1 mM, 46% by 1 mM, and 92% by 10 mM iodoacetate, but the pathway involved is not known (Hoshino, 1959). Oxidation of steroids is resistant to iodoacetate (Levy and Talalay, 1959).

**Biosynthesis of Lipids**

Determinations of tissue lipid changes with time as affected by iodoacetate reflect disturbances in the balance between lipid synthesis and catabolism. The lipid content of pea stems is initially 8.95% (dry weight), after incubation with auxin is 6.18%, and with auxin and iodoacetate (at a concentration inhibiting growth 50%) is 7.44% (Christiansen and Thimann, 1950 b). Is iodoacetate in some way accelerating synthesis of lipid or is it reducing the utilization of lipid? In this instance lipid is being converted to carbohydrate and iodoacetate inhibits this. It is unlikely that the iodoacetate stimulates lipid synthesis in any case, and all the evidence points to a rather strong depression. Lipid formation in yeast, for example, is inhibited 24% by 0.01 mM iodoacetate (Hoffmann-Ostenhof and Kriz, 1949 b).
The most reliable work is the demonstration of the inhibition of incorporation of C\textsuperscript{14} into lipids. Thus the formation of labeled fatty acids from acetate-1-C\textsuperscript{14} in various tumors is inhibited by 0.54 mM iodoacetate, although to very different degrees, and never as much as the incorporation into cholesterol, proteins, or CO\textsubscript{2} (van Vals and Emmelot, 1957). The incorporation of label from glucose-C\textsuperscript{14} should be more potently inhibited, and in mammary gland 0.1 mM iodoacetate reduces lipid labeling around 65\% (McLean, 1962). Lynen (1959) showed that the reaction of acetyl-CoA with CO\textsubscript{2} and ATP, catalyzed by a yeast enzyme, is blocked by 10 mM iodoacetamide at the level of malonyl-CoA, and concluded that iodoacetamide would block synthesis of lipid from malonyl-CoA (the unfortunately high concentration must be noted). Vagelos and Alberts (1960) also showed that the enzyme catalyzing the reaction

\[
\text{Malonyl-CoA} + R–\text{CO}–\text{CoA} \leftrightarrow \text{CO}_2 + R–\text{CO}–\text{CH}_2–\text{CO}–\text{CoA} + \text{CoA}
\]

in Clostridium klyveri is quite strongly inhibited by iodoacetate and iodoacetamide. Thus McLean suggested that the effects of iodoacetate on mammary gland may be primarily on the utilization of two-carbon units for lipid synthesis rather than on the formation of the two-carbon units.

Sterol synthesis is very sensitive to iodoacetate. In microsomal and supernate fractions from rat liver the incorporation of acetate-1-C\textsuperscript{14} into cholesterol is inhibited 53\% by 0.02 mM iodoacetate (Bucher and McGarrah, 1956). The authors felt that the synthesis is dependent on glycolysis for the supply of ATP and that iodoacetate blocks here, but did not show that ATP would counteract the inhibition. The formation of corticoids in rat adrenal when stimulated by ACTH is inhibited some 70\% by 1 mM iodoacetate and this is not altered by pyruvate (Schönbaum et al., 1956). Since the cycle is not important and the pentose-P pathway is very active, it appears that sterol synthesis is affected directly. Later steps in sterol genesis may be sensitive to iodoacetate. Thus the incorporation of mevalonate-C\textsuperscript{14} in Lactobacillus casei is inhibited 54\% by 1 mM iodoacetate (Thorne and Kodicek, 1962), the incorporation of 3P-isopentenyl-PP into lycopene in tomato homogenates is blocked completely by 5.4 mM iodoacetamide (Varma and Chichester, 1962), the conversion of farnesyl-PP to squalene in liver microsomes is inhibited 12\% by 1 mM iodoacetamide (Anderson et al., 1960), and the formation of labeled sterol from squalene-C\textsuperscript{14} in liver is reduced 46\% by 0.42 mM iodoacetamide (Goodman, 1961). Thus there is sufficient evidence that these inhibitors may depress sterol synthesis at several points. Once the sterols are formed, their interconversions are probably not sensitive to iodoacetate; at least oxidations and hydroxylations are quite resistant.

The synthesis of phospholipids has frequently been studied by measuring the incorporation of P\textsuperscript{32} into various fractions. The problem with cellular
preparations is whether iodoacetate is blocking uptake of $P^{32}$, the actual incorporation of $P^{32}$, or some other pathway such as glycolysis, the decreased formation of lipid-$P^{32}$ being only a reflection of a depression of precursors. Thus in cat brain slices 1 mM iodoacetate inhibits 86% the formation of lipid-$P^{32}$ but this could be on glycolysis rather than a specific effect on phospholipid synthesis (Strickland, 1954). In *Mycobacterium tuberculosis* the incorporation of $P^{32}$ into lipid-$P^{32}$ is depressed around 50% by 1 mM iodoacetate with fumarate and pyruvate as substrates, the $O_2$ uptake being unaffected, so that here a nonglycolytic site is likely (Tanaka, 1960). The incorporation in subcellular preparations varies with the organism or tissue as well as the conditions of the experiment. Thus in brain dispersions, iodoacetate inhibits weakly if pyruvate is the substrate (Dawson, 1953) but very strongly if glucose is the substrate (McMurray et al., 1957 a). In the latter case, iodoacetate at 0.01 mM depresses the incorporation into phospholipid 40% aerobically and 72% anaerobically. There are two systems for the synthesis of phospholipids, one dependent on glycolysis and the other on the cycle in mitochondria (McMurray et al., 1957 b), and the latter is not inhibited by 0.01 mM iodoacetate. Little work on the incorporation of phosphorylated intermediates has been done but, in rat brain extracts, 1 mM iodoacetate inhibits choline-$P^{32} \rightarrow$ lipid-$P^{32}$ 69% and $\alpha$-glycerol-$P^{32} \rightarrow$ lipid-$P^{32}$ 96% (McMurray et al., 1957 c). The incorporation of acetate-$1-C^{14}$ into phospholipids in liver slices is completely blocked by 1 mM iodoacetate (Kline and DeLuca, 1956). Although the evidence is not complete, it is reasonable that iodoacetate can exert some direct effect on the later steps in the synthesis of phospholipids, but in other instances the action is mainly through glycolytic depression. In the biosynthesis of lecithin, however, the formation of choline is not sensitive to iodoacetate (Bremer and Greenberg, 1961).

### EFFECTS ON PROTEIN AND AMINO ACID METABOLISM

The effects of iodoacetate on proteolytic enzymes have been discussed (page 39) and it is evident that certain intracellular cathepsins and proteinases are quite sensitive while others are very resistant. Some may well be as sensitive as 3-PGDH and, particularly in experiments involving the nutrition and growth of microorganisms, these effects must be taken into account. There is presumably a balance between protein synthesis and breakdown, and this may be disturbed by iodoacetate in either direction since, as we shall see, protein formation is quite sensitive to iodoacetate.

Ammonia formation and release from tissues interested the early workers, inasmuch as to most they appeared to reflect protein or amino acid catabolism. It is now known that ammonia can arise from a number of reactions: the reduction of nitrate and nitrite, the deamidation of glutamine...
and asparagine, and the deamination of urea, amino acids, hexosamines, nucleotides, nucleosides, and amino purines. The results are thus not easy to interpret. Embden (1930; Embden and Norpoth, 1931) associated ammonia formation with the contracture induced by bromoacetate in frog muscles, since the ammonia content increases markedly in stimulated bromoacetate-treated muscles but not in those merely stimulated or incubated with bromoacetate. Similar results were obtained by Mozolowski et al. (1931) who showed that ammonia formation occurs maximally, not when the muscle is most active but when it begins to fail and go into rigor, at the time creatine-P has dropped to a low level. In mammalian tissues the situation seems to be different, in that Barker et al. (1939) obtained only inhibition of ammonia formation, this being especially marked in brain, which has a high ammonia production, 0.054 mM iodoacetate inhibiting 83%. Weil-Malherbe and Green (1955a) did not find such potent inhibition in guinea pig brain, but observed that ammonia formation is suppressed by glucose due to synthesis of amides. This then introduces another aspect into the problem: if glucose is forming $C_3$ and $C_1$ acids through the EM pathway and these acids are involved in the uptake of what ammonia is formed, inhibition of the EM pathway would increase the free ammonia, as Embden observed. However, it does not correlate with the effects in brain, where ammonia formation is depressed by inhibitors of electron transport, uncouplers of oxidative phosphorylation, and anoxia. Weil-Malherbe and Green felt that the ammonia arises mainly in reactions associated with proteolysis. Takagaki et al. (1957) clarified the picture somewhat by showing that brain slices endogenously form ammonia with a simultaneous decrease in glutamate, more than half the endogenous respiration being accounted for by the glutamate disappearance, which glucose inhibits. Iodoacetate at 0.05 mM inhibits 13% the endogenous ammonia formation, and this is likely to be on the enzyme oxidatively deaminating glutamate.

Essentially nothing is known of the effects of iodoacetate on the various long synthetic pathways for amino acids, but certain simple reactions have been studied. If guinea pig brain slices are incubated with glucose-$C^{14}$, some of the label appears in amino acids, of which glutamate is the most important (Tsukada et al., 1958). Iodoacetate at 0.1 mM completely inhibits this, but of course the action here could be entirely on the glycolytic pathway. The formation of alanine from ammonia and pyruvate in Bacillus subtilis (Fairhurst et al., 1956) and liver mitochondria (Berezovskaya, 1960) is also rather potently inhibited.

The synthesis of protein requires not only the necessary amino acids (either synthesized or transported into the cell) but a source of ATP; it is, therefore, quite susceptible to inhibition by iodoacetate. The transport of amino acids into the cell is often reduced by iodoacetate, so this must be one of the major sites for the reduction of over-all protein synthesis. The
transport may be blocked either directly or through ATP depletion. Thus in *Staphylococcus aureus* the uptake of glutamate requires energy and occurs when glucose is fermented (Gale, 1951). The inhibition by iodoacetate here could be indirect. The uptakes into bacteria and plant tissue (Birt and Hird, 1958) are well depressed by 2,4-dinitrophenol, indicating the importance of ATP. On this basis one would expect the incorporation of amino acids into proteins to be strongly inhibited by iodoacetate, but in only one case, that of pea stems where synthesis of plasma proteins is reduced almost completely by 0.6 mM iodoacetate (Christiansen and Thimann, 1950 c), has this been found to be true. In reticulocytes the incorporation of several amino acids is inhibited only moderately by 2 mM iodoacetate (Borsook et al., 1952), and in bull spermatozoa there is essentially no effect of 5 mM iodoacetate (Bhargava et al., 1959). Moderate inhibition is seen in the spermatozoa anaerobically, which seems to point to an interference with the supply of energy. The incorporation at 90 min is depressed 28% aerobically and 52% anaerobically (Abraham and Bhargava, 1963). The incorporation of C14-labeled amino acids into protein anaerobically in rat liver homogenate in the presence of creatine-P to generate ATP is inhibited 7% by 0.05 mM, 54% by 0.5 mM, and 94% by 5 mM iodoacetate (Zamecnik and Keller, 1954) and this certainly points to a direct action on some step in the synthesis. The activation of amino acids by ATP and the appropriate liver enzyme, preparatory to protein synthesis, is inhibited only 23% by 1 mM iodoacetate, so that this is probably not an important site of action.

The effects of iodoacetate on some of the enzymes involved in amino acid metabolism are shown in Table 1-24 and, with certain exceptions, it may be noted that as a class these enzymes are fairly resistant. Exceptions are mammalian L-amino acid oxidase, certain decarboxylases, prolidase, and serine deaminase. It is interesting that the transaminases, which are generally SH enzymes, are not readily inhibited by iodoacetate.

In connection with amino acid metabolism, it may be mentioned that the urea cycle can be depressed by iodoacetate through ATP depletion, as postulated by Cohen and Hayano (1946) to explain the moderate inhibition (74% by 10 mM) on the conversion of citrulline to arginine in liver homogenate. On the other hand, the reactions arginine → citrulline (Korzenovsky, 1955) and citrulline → ornithine (Korzenovsky and Werkman, 1953) are quite resistant to iodoacetate. The arsenolysis of citrulline to form ornithine catalyzed by an enzyme from *Streptococcus faecalis* is, however, inhibited almost completely by 1.67 mM iodoacetate (Slade, 1955). Modifications in urea formation by iodoacetate might also occur through effects on amino acid metabolism. There is little evidence of direct potent inhibition by iodoacetate on the urea cycle itself.

Several enzymes involved in nitrogen fixation and nitrate reduction have been examined (Table 1-25), but no studies on the general effects of iodo-
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Iodoacetate (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
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<td>L-Alanine oxidative deaminase</td>
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<td>Nohara and Ogata (1959)</td>
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<td>Inh.</td>
<td>Sutton and King (1959)</td>
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<td>—</td>
<td>0</td>
<td>Klein and Kamin (1941)</td>
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<tr>
<td></td>
<td>Rat kidney</td>
<td>5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>Singer and Barron (1945)</td>
</tr>
<tr>
<td>L-Amino acid oxidase</td>
<td><em>Proteus vulgaris</em></td>
<td>10</td>
<td>0</td>
<td>Stumpf and Green (1944)</td>
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<td>10</td>
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<td>Aspis venom</td>
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<td>Zeller and Maritz (1945)</td>
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<td>75</td>
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<td>Horse liver</td>
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<td>0</td>
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<td>Greenberg et al. (1956)</td>
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<td><em>Mycobacterium smegmatis</em></td>
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<td>Guinea pig serum</td>
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<td>Tower et al. (1963)</td>
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<td>Source</td>
<td>Iodoacetate (mM)</td>
<td>% Inhibition</td>
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<td>20</td>
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<td>Ichihara <em>et al.</em> (1955)</td>
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<td>10</td>
<td>Black and Wright (1955)</td>
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<td></td>
<td></td>
<td>10</td>
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<td>Lowenstein and Cohen (1956)</td>
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<td>Ohigashi <em>et al.</em> (1952)</td>
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<td>Pea epicotyls</td>
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<td>Cruickshank and Isherwood</td>
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<td></td>
<td></td>
<td></td>
<td>(1958)</td>
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<td>Cohen (1939)</td>
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<td>Heart</td>
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<td>Enzyme</td>
<td>Source</td>
<td>Enzyme Inhibition (%)</td>
<td>Ref.</td>
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<td>Baxter and Roberts (1958)</td>
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<td>T'ing-seng (1961 b)</td>
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<td>Cauliflower</td>
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<td>Ellis and Davies (1961)</td>
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<td>Williams and Watson (1947)</td>
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<td>Fonnum &lt;i&gt;et al.&lt;/i&gt; (1964)</td>
<td></td>
</tr>
<tr>
<td>Tyrosine:α-ketoglutarate</td>
<td>Rat liver</td>
<td>1</td>
<td>Kenney (1959)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dog liver</td>
<td>10</td>
<td>Canellakis and Cohen (1956 a)</td>
<td></td>
</tr>
<tr>
<td>Unspecified</td>
<td>Leishmania donovani</td>
<td>1</td>
<td>Chatterjee and Ghosh (1957)</td>
<td></td>
</tr>
<tr>
<td>Tryptophanase</td>
<td>Escherichia coli</td>
<td>1, 5, 10</td>
<td>Wada &lt;i&gt;et al.&lt;/i&gt; (1958)</td>
<td></td>
</tr>
<tr>
<td>β-Tyrosinase</td>
<td>Escherichia coli</td>
<td>1, 2, 10</td>
<td>Ichihara &lt;i&gt;et al.&lt;/i&gt; (1956)</td>
<td></td>
</tr>
<tr>
<td>Valine decarboxylase</td>
<td>Proteus vulgaris</td>
<td>1</td>
<td>Ekladius &lt;i&gt;et al.&lt;/i&gt; (1957)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Inh = inhibition of unspecified degree.
<sup>b</sup> Iodoacetamide.
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Iodoacetate (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydroxylamine:NADH oxidoreductase</td>
<td>Soybean leaves</td>
<td>1</td>
<td>0</td>
<td>Roussos and Nason (1960)</td>
</tr>
<tr>
<td></td>
<td>Chicken liver</td>
<td>2.5</td>
<td>89</td>
<td>Osajima and Yamafuji (1962)</td>
</tr>
<tr>
<td></td>
<td>Potato</td>
<td>1</td>
<td>63</td>
<td>Franke and Schumann (1942)</td>
</tr>
<tr>
<td></td>
<td><em>Achromobacter fischeri</em></td>
<td>20</td>
<td>0</td>
<td>Sadana and McElroy (1957)</td>
</tr>
<tr>
<td>Nitrate:NADH oxidoreductase</td>
<td><em>Rhizobium japonicum</em></td>
<td>20</td>
<td>0</td>
<td>Cheniae and Evans (1959)</td>
</tr>
<tr>
<td></td>
<td>Chicken liver</td>
<td>2.5</td>
<td>93</td>
<td>Osajima and Yamafuji (1962)</td>
</tr>
<tr>
<td>Nitrate:NADPH oxidoreductase</td>
<td><em>Neurospora crassa</em></td>
<td>10</td>
<td>0</td>
<td>Nason and Evans (1953)</td>
</tr>
<tr>
<td>Nitrate reductase</td>
<td>Wheat roots</td>
<td>0.001</td>
<td>5</td>
<td>Nance (1950)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>77</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Clostridium welchii</em></td>
<td>0.1</td>
<td>50</td>
<td>Katsura <em>et al.</em> (1954)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Nitrate:succinate oxidoreductase</td>
<td><em>Rhizobium japonicum</em></td>
<td>20</td>
<td>0</td>
<td>Cheniae and Evans (1959)</td>
</tr>
<tr>
<td>Nitrite:lactate oxidoreductase</td>
<td>Denitrifying bacterium</td>
<td>10</td>
<td>50</td>
<td>Iwasaki <em>et al.</em> (1956)</td>
</tr>
<tr>
<td>Nitrite:NADH oxidoreductase</td>
<td>Soybean leaves</td>
<td>1</td>
<td>20</td>
<td>Roussos and Nason (1960)</td>
</tr>
<tr>
<td></td>
<td>Chicken liver</td>
<td>2.5</td>
<td>68</td>
<td>Osajima and Yamafuji (1962)</td>
</tr>
<tr>
<td>Nitrite reductase</td>
<td><em>Nitrobacter agilis</em></td>
<td>10</td>
<td>18</td>
<td>Alem and Alexander (1958)</td>
</tr>
<tr>
<td>Nitroreductase</td>
<td>Rat liver</td>
<td>10</td>
<td>100</td>
<td>Honda <em>et al.</em> (1956)</td>
</tr>
<tr>
<td></td>
<td>Pig liver</td>
<td>5</td>
<td>75</td>
<td>Otsuka (1961)</td>
</tr>
<tr>
<td>Nitrosoreductase</td>
<td>Pig liver</td>
<td>5</td>
<td>34</td>
<td>Otsuka (1961)</td>
</tr>
</tbody>
</table>
acetate on the patterns of these metabolic systems have been reported. It is clear that nitrate reduction must be inhibited fairly potently by iodoacetate. Sulfur oxidation by *Thiobacillus thiooxidans* is also markedly depressed by iodoacetate, 60% at 0.01 mM and almost completely at 0.1 mM (Vogler et al., 1942), growth being simultaneously abolished. Very similar results were obtained by Iwatsuka et al. (1962), an iodoacetate concentration as low as 0.001 mM inhibiting 15%, but the CO₂ fixation in the presence of sulfide is even more sensitive, being reduced 82% at 0.001 mM. The incorporation of sulfate-$S^{35}$ into *Chlorella pyrenoidosa* is inhibited when iodoacetamide is added before the sulfate; when the iodoacetamide is added 5 min after the sulfate (which is a rather strange way to examine the effect of an inhibitor) the total uptake is unchanged but the pattern of incorporation is altered (Schiff, 1959). Sulfated polysaccharide labeling is reduced and more labeling occurs in lower molecular weight compounds.

**EFFECTS ON PORPHYRIN SYNTHESIS**

The synthesis of protoporphyrin from glycine and α-ketoglutarate in chicken erythrocytes is inhibited 15% by 1 mM iodoacetamide and 63% by 10 mM iodoacetamide, which indicates this part of the pathway to be fairly resistant (Granick, 1958). The initial reactions of the synthesis, the formation of δ-aminolevulinate from glycine, succinyl-CoA, and pyridoxal-P, are not affected by 3.5 mM iodoacetamide (Gibson et al., 1958), so that a more distal site must be visualized. This may well be the condensation of δ-aminolevulinate to form porphobilinogen, since this is inhibited strongly by 1 mM iodoacetamide (Gibson et al., 1955). The further condensation of porphobilinogen to coproporphyrin or protoporphyrin is not inhibited very markedly by up to 10 mM iodoacetate, although there is some diversion of the path from coproporphyrin to protoporphyrin (Rimington and Tooth, 1961), and the formation of uroporphyrinogen III from porphobilinogen is only slightly depressed (Lockwood and Benson, 1960). The decarboxylation of uroporphyrinogen to coproporphyrinogen is, however, well inhibited by 1.2 mM iodoacetamide (Mauzerall and Granick, 1958), so that where this reaction is significant it may be a susceptible site for inhibition. The transmethylation from *S*-adenosylmethionine to Mg-protoporphyrin to form a methyl ester is actually stimulated slightly by 1–2 mM iodoacetate and iodoacetamide (Gibson et al., 1963). The final incorporation of iron into heme is moderately sensitive to iodoacetate in intact reticulocytes (59% inhibition by 1 mM) (Yoshiba et al., 1958), but in hemolysates from chicken erythrocytes the inhibition is much less (9% by 1 mM) (Kagawa et al., 1959), indicating that in the former case the inhibition may be through ATP depletion (2,4-dinitrophenol inhibits potently). From the limited evidence one would conclude that in the synthetic pathway the most sensitive
site is the formation of porphobilinogen, but that in cells some depression may derive from ATP depletion, or possibly even the availability of succinyl-CoA. Effects on hematopoiesis in intact animals must also be taken into account.

**EFFECTS ON PHOTOSYNTHESIS**

The effects of iodoacetate and iodoacetamide on photosynthesis have been studied intensively since the original demonstration by Kohn in 1935 that a very potent inhibition is exerted, and these inhibitors have proved valuable in the analyses of the photosynthetic pathways and mechanisms. Results have often been conflicting and the site of the block has evaded certain localization, but the recent techniques for tracing the distribution of C¹⁴ throughout the photosynthetic carbon-reduction cycle (Calvin cycle) following illumination have provided the means for obtaining some understanding of how these inhibitors act. Photosynthesis may be roughly divided into two phases. The *light reactions* comprise the photon absorption by chlorophyll, the initiation of electron flow, the formation of ATP, the reduction of NAD(P) to NAD(P)H,* and the evolution of O₂. The *dark reactions* include the fixation of CO₂ and the complex sequence of chemical reactions whereby carbohydrate is synthesized. An inhibitor of total photosynthesis may act primarily on either the light or dark reactions. In the case of iodoacetate the important site of attack is within the photosynthetic carbon cycle, and the light reactions are significantly affected only at relatively high concentrations.

That the inhibition is primarily on the dark reactions was indicated in the early work of Kohn (1935) on Chlorella pyrenoidosa. Although O₂ evolution was measured, it was assumed that the rate is limited by the dark reactions (Blackman reaction) with continuous illumination, and by the light reactions (photochemical reaction) with flashing light. Inasmuch as inhibition in continuous light is greater than in intermittent light, Kohn concluded that the inhibition is exerted on the dark reactions (it may be noted that his definition of dark reactions does not exactly coincide with modern terminology). Iodoacetamide at low concentrations (0.006–0.01 mM) stimulates photosynthesis as much as 30%, an effect assumed to be on the photochemical mechanism. No reaction of iodoacetate with chlorophyll can be spectroscopically detected. Finally, Kohn noted the marked differential effect on photosynthesis and respiration, it being possible to inhibit the former while respiration is unaffected or actually stimulated, an observation repeatedly reported and considered by some to be of significance.

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* The abbreviation NAD(P) will be used to designate both NAD and NADP in processes where either or both may be involved.
Photochemical Light Reactions and Phosphorylation

The Hill reaction (photochemical decomposition of water accompanied by evolution of $O_2$ and reduction of some substance such as quinone or an indo-phenol) is not very sensitive to iodoacetate (IA). It requires 10 mM to inhibit 50% in spinach chloroplasts (Maidowall, 1949) while the Hill reaction in Chlorella is not affected by 2.7–7.4 mM iodoacetamide (IAM) (Fraser, 1954; Whittingham, 1956), although 20 mM may inhibit nearly completely (Ehrmantraut and Rabinowitch, 1952). Since photosynthesis is strongly inhibited at concentrations far below these, one can eliminate the initial light reaction as an important site for the inhibition. The reduction of NADP is likewise resistant, no inhibition being observed in spinach grana at 0.6 mM iodoacetate (San Pietro and Lang, 1958) or in spinach chloroplasts at 0.05 mM iodoacetamide (Gibbs and Calo, 1960 a), at which concentrations the fixation of $CO_2$ is markedly depressed. In fact, iodoacetamide at 10 mM and 20 mM inhibits only 30% and 40%, respectively (J.S.C. Wessels, 1959). Photosynthetic phosphorylation seems to be the least sensitive of all the light reactions. Iodoacetamide at 10 mM has no effect in spinach chloroplasts (Arnon et al., 1956; Avron et al., 1957; J.S.C. Wessels, 1959) or in dahlia, datura, and tomato leaves (Massini, 1957). These results, taken with the failure of iodoacetamide to inhibit the burst in $O_2$ evolution upon illumination (Whittingham, 1956), all indicate strongly that the light reactions are resistant to these inhibitors and, at low concentrations inhibiting total photosynthesis, no effect on these reactions is to be expected.

Photosynthetic Carbon Cycle (Dark Reactions)

Total photosynthetic formation of carbohydrate as influenced by iodoacetate has seldom been measured and where it has been the results are difficult to interpret, although incorporation of $C^{14}O_2$ into various fractions has been studied thoroughly (page 158). Photosynthetic activity in most instances has been determined by measuring $CO_2$ utilization. The results are variable because in cellular preparations the penetration of iodoacetate may limit its action. For this reason, iodoacetamide has often been used and generally is more potent than iodoacetate in the pH range above 6. Extremely potent inhibition has been observed in some cells; indeed, the inhibition is often as marked as that of the EM pathway, as seen in the accompanying tabulation. The much smaller inhibition reported by Arnon et al. (1956) in spinach chloroplasts — 17% by 1 mM and 79% by 5 mM iodoacetamide — is unexplained. Perhaps under certain conditions there are two or more pathways for $CO_2$ fixation, only the photosynthetic one being extremely sensitive.

Following illumination for 4–30 sec in the presence of $C^{14}O_2$ the first stable intermediate (i.e., with the highest specific activity) was found to be 3-P-
<table>
<thead>
<tr>
<th>Preparation</th>
<th>Inhibitor</th>
<th>Concentration (mM)</th>
<th>% Inhibition of CO₂ fixation</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chlorella pyrenoidosa</em></td>
<td>IAM</td>
<td>0.15</td>
<td>90</td>
<td>Calvin <em>et al.</em> (1951)</td>
</tr>
<tr>
<td></td>
<td>IAM</td>
<td>0.77</td>
<td>90-100</td>
<td>Holzer (1954)</td>
</tr>
<tr>
<td></td>
<td>IA</td>
<td>0.001</td>
<td>75</td>
<td>Kandler <em>et al.</em> (1961)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01</td>
<td>98</td>
<td>Fraser (1954)</td>
</tr>
<tr>
<td>Spinach chloroplasts</td>
<td>IAM</td>
<td>0.014</td>
<td>50</td>
<td>Calo and Gibbs (1960)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.005</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>99</td>
<td></td>
</tr>
</tbody>
</table>

glycerate. Since formation of hexoses could occur through reduction of the 3-P-glycerate [with photochemically formed NAD(P)H and ATP] by a reversal of the EM pathway, it was evident that iodoacetate might inhibit photosynthesis by blocking 3-PGDH, i.e., by the same mechanism as the inhibition of glycolysis. If this were the case, one might expect some accumulation of 3-P-glycerate in the presence of iodoacetate. However, Calvin *et al.* (1951) reported that when 0.15 mM iodoacetamide has inhibited the uptake of CO₂ by 90% in *Chlorella*, the synthesis of sucrose is not decreased and no 3-P-glycerate accumulates. This led them to postulate that iodoacetamide is acting on an enzyme other than 3-PGDH or the formation of sucrose does not involve reversal of the EM pathway. However, Arnon (1952) reexamined the data obtained by Stepka (1951) and claimed that if the incubation of the cells with the inhibitor is longer, inhibition of sucrose formation and accumulation of 3-P-glycerate occur, the data obtained above having been on preparations not sufficiently inhibited. Nevertheless, it is still difficult to understand how sucrose synthesis can be unaffected while CO₂ utilization is inhibited 90%, which means that the formation of the sucrose from the CO₂ which is taken up is increased about 10 fold. One would normally interpret these data as indicating iodoacetamide to block various pathways for CO₂ incorporation, allowing it to be channeled into sucrose. In order to understand the effects of these inhibitors we must turn to more recent investigations of the changes in the patterns of labeling.

**Localization of the Site of Action of Iodoacetate and Iodoacetamide on Photosynthesis**

The effects of iodoacetate or iodoacetamide on the patterns of incorporation of C14O₂ during periods of illumination in several plants are shown in Tables 1-26 to 1-29, and on the incorporation of inorganic P32 in Table 1-30. The relatively high concentrations of iodoacetate used in the study of sugar
Table 1-26<sup>a</sup>

Effect of 0.15 mM Iodoacetamide on Patterns of C<sup>14</sup> Incorporation in Tobacco Leaves

<table>
<thead>
<tr>
<th>Compound</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Illumination 4 sec</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>% C&lt;sup&gt;14&lt;/sup&gt; fixed</td>
<td>Specific activity</td>
<td>% C&lt;sup&gt;14&lt;/sup&gt; fixed</td>
<td>Specific activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>IAM</td>
<td>Control</td>
<td>IAM</td>
<td>Control</td>
</tr>
<tr>
<td>Phosphoglycerate</td>
<td>7</td>
<td>2</td>
<td>128</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>Fructose-1,6-diP</td>
<td>6</td>
<td>7</td>
<td>85</td>
<td>28</td>
<td>5</td>
</tr>
<tr>
<td>Glucose-P</td>
<td>2</td>
<td>4</td>
<td>36</td>
<td>19</td>
<td>13</td>
</tr>
<tr>
<td>Phosphate esters&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>23</td>
<td>30</td>
<td>68</td>
<td>—</td>
</tr>
<tr>
<td>Malate</td>
<td>18</td>
<td>14</td>
<td>—</td>
<td>—</td>
<td>14</td>
</tr>
<tr>
<td>Glycine</td>
<td>2</td>
<td>—</td>
<td>5</td>
<td>—</td>
<td>5</td>
</tr>
<tr>
<td>Alanine</td>
<td>41</td>
<td>36</td>
<td>21.6</td>
<td>26</td>
<td>24</td>
</tr>
<tr>
<td>Serine</td>
<td>4</td>
<td>7</td>
<td>10.3</td>
<td>—</td>
<td>7</td>
</tr>
<tr>
<td>Aspartate</td>
<td>9</td>
<td>2</td>
<td>4.7</td>
<td>4.5</td>
<td>6</td>
</tr>
<tr>
<td>Glutamate</td>
<td>2</td>
<td>—</td>
<td>3.8</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

<sup>a</sup> From Newburgh and Burris (1954).

<sup>b</sup> Includes fructose phosphates and gluconate-6-P.
TABLE 1-27

**Effect of Iodoacetate on Patterns of C\textsuperscript{14} Incorporation in Sugar Beet and Soybean Leaves\textsuperscript{a}**

<table>
<thead>
<tr>
<th></th>
<th>Per cent total ethanol-soluble C\textsuperscript{14}</th>
<th>Sugar beet leaves with iodoacetate</th>
<th>Soybean leaves with iodoacetate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>5 \text{mM}</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>10 \text{min}</td>
</tr>
<tr>
<td>Phosphoglycerate</td>
<td></td>
<td>15</td>
<td>40</td>
</tr>
<tr>
<td>Hexose phosphates</td>
<td></td>
<td>24</td>
<td>14</td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>Glycerate</td>
<td></td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>Phosphoenolpyruvate</td>
<td></td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Malate</td>
<td></td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Glycine</td>
<td></td>
<td>8</td>
<td>Trace</td>
</tr>
<tr>
<td>Alanine</td>
<td></td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Serine</td>
<td></td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Aspartate</td>
<td></td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>Relative C\textsuperscript{14} assimilated</td>
<td>100</td>
<td>70</td>
<td>60</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The times given for each concentration of iodoacetate refer to the durations of petiolar uptake of the inhibitor. The illumination period was 60 sec. (From Mortimer, 1960.)
Table 1-28

Effect of Iodoacetate on Patterns of C\textsuperscript{14} Incorporation in *Chlorella*\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th><em>Chlorella pyrenoidosa</em></th>
<th></th>
<th><em>Chlorella ellipsoidea</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>IA 0.001 mM</td>
<td>IA 0.01 mM</td>
<td>Control</td>
</tr>
<tr>
<td>Insoluble fraction (polysaccharide)</td>
<td>44</td>
<td>20</td>
<td>24</td>
<td>64</td>
</tr>
<tr>
<td>Soluble fraction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate esters</td>
<td>56</td>
<td>80</td>
<td>76</td>
<td>36</td>
</tr>
<tr>
<td>Sucrose</td>
<td>3</td>
<td>23.6</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Malate</td>
<td>48</td>
<td>25</td>
<td>8</td>
<td>25.5</td>
</tr>
<tr>
<td>Glycine + serine</td>
<td>0.8</td>
<td>4.0</td>
<td>12</td>
<td>1.6</td>
</tr>
<tr>
<td>Alanine</td>
<td>1</td>
<td>9.5</td>
<td>25</td>
<td>1</td>
</tr>
<tr>
<td>Aspartate</td>
<td>0.4</td>
<td>5.1</td>
<td>4</td>
<td>0.25</td>
</tr>
<tr>
<td>Glutamate</td>
<td>1</td>
<td>9</td>
<td>9</td>
<td>0.6</td>
</tr>
<tr>
<td>Citrulline</td>
<td>0.1</td>
<td>1.7</td>
<td>13</td>
<td>1.4</td>
</tr>
<tr>
<td>Unaccounted for</td>
<td>1.6</td>
<td>1.3</td>
<td>1</td>
<td>1.5</td>
</tr>
<tr>
<td>Relative C\textsuperscript{14} assimilated</td>
<td>100</td>
<td>29</td>
<td>2</td>
<td>100</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The cell suspensions were incubated with iodoacetate for 2 hr and the fixation of C\textsuperscript{14}O\textsubscript{2} was over a period of 1 hr. (From Kandler \textit{et al.}, 1961.)
Table 1-29

**Effect of Iodoacetate on Pattern of C\textsuperscript{14} Incorporation in Chlorella ellipsoidea\textsuperscript{a}**

<table>
<thead>
<tr>
<th></th>
<th>Per cent of total C\textsuperscript{14} taken up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Insoluble fraction</td>
<td></td>
</tr>
<tr>
<td>(polysaccharide)</td>
<td>29.8</td>
</tr>
<tr>
<td>Soluble fraction</td>
<td>70.2</td>
</tr>
<tr>
<td>Phosphoglycerate</td>
<td>23.0</td>
</tr>
<tr>
<td>Fructose monophosphates</td>
<td>5.5</td>
</tr>
<tr>
<td>Glucose phosphate + heptose phosphates</td>
<td>21.0</td>
</tr>
<tr>
<td>Pentose phosphates</td>
<td>5.0</td>
</tr>
<tr>
<td>Fructose-1,6-diP</td>
<td>5.6</td>
</tr>
<tr>
<td>Phosphoenolpyruvate</td>
<td>2.8</td>
</tr>
<tr>
<td>Sucrose</td>
<td>1.5</td>
</tr>
<tr>
<td>Amino acids</td>
<td>9.1</td>
</tr>
<tr>
<td>Organic acids</td>
<td>1.5</td>
</tr>
<tr>
<td>Relative C\textsuperscript{14} assimilated</td>
<td>100</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The cell suspensions were incubated with iodoacetate for 2 hr and the fixation of C\textsuperscript{14}O\textsubscript{2} was over a period of 60 sec. (From Kandler et al., 1961.)

Table 1-30

**Effect of Iodoacetate on Pattern of P\textsuperscript{32} Incorporation in Helodea densa\textsuperscript{a}**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Per cent of total P\textsuperscript{32} taken up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dark</td>
</tr>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>3-P-Glycerate</td>
<td>1.12</td>
</tr>
<tr>
<td>Phosphoenolpyruvate</td>
<td>0.315</td>
</tr>
<tr>
<td>Fructose-1,6-diP</td>
<td>0.145</td>
</tr>
<tr>
<td>Fructose-6-P</td>
<td>0.805</td>
</tr>
<tr>
<td>Glucose-6-P</td>
<td>1.465</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The cells were preincubated with iodoacetate for 9 min, the period of illumination was 10 min, and the period for uptake of P\textsuperscript{32} was 2 min. (From Simonis and Weichart, 1958.)
beet and soybean leaves (Table 1-27) were necessary because the inhibitor solutions were allowed to enter the leaf through the petiole, and it is likely that even in 15 min, the longest period used, the iodoacetate was not completely distributed in the leaves. It should be noted that the figures in Tables 1-27 to 1-29 refer to the percent of the total C\textsuperscript{14} taken up and not the actual amount of incorporation into the various fractions; to obtain these latter values, it is necessary to take into account the change in the total incorporation, the data for which are to be found in the last row of each table. For example, it might be concluded that sucrose formation in <i>Chlorella ellipsoida</i> is not significantly changed by iodoacetate up to 0.01 mM (Table 1-29), but yet there is 65% inhibition at 0.003 mM and 91% at 0.01 mM iodoacetate; the figures show that the total incorporation of CO\textsubscript{2} is depressed but of that incorporated the same fraction goes to sucrose.

Summarizing the major changes to be seen in these tables, we can draw the following general conclusions.

(A) \textit{CO\textsubscript{2} incorporation}. This is quite potently inhibited by even very low concentrations of the inhibitors.

(B) \textit{Sucrose or polysaccharide synthesis}. Total synthesis is always strongly depressed, but the pattern of the initial incorporation is unchanged; however, after longer periods (1 hr in Table 1-28) relatively less of the C\textsuperscript{14} taken up goes to synthesis.

(C) \textit{Hexose phosphates}. Incorporation into this fraction is decreased, whether measured by C\textsuperscript{14} or P\textsuperscript{32} (Table 1-30), although in long experiments they may accumulate somewhat as they do in the dark.

(D) \textit{3-Phosphoglycerate}. The results here are more variable; in general there is relatively more of the C\textsuperscript{14} or P\textsuperscript{32} utilized found in this compound. At the lowest concentrations of iodoacetate there is a tendency for absolute incorporation to rise, indicating some accumulation, but at higher concentrations this is depressed. There is no evidence for marked accumulation.

(E) \textit{Malate}. The fraction of the C\textsuperscript{14} appearing in malate increases and the total incorporation is usually unchanged or somewhat depressed.

(F) \textit{Amino acids}. Relatively more of the label is found in amino acids, showing some diversion of the pathway toward amino acids, but the total synthesis is well depressed, except at the lowest concentrations of iodoacetate in <i>Chlorella</i>.

If one assumes the reversal of the EM pathway and the photosynthetic carbon cycle to operate as originally postulated by Calvin, the inhibition by iodoacetate can be attributed to a block of 3-PGDH, depressing both the carbon cycle and the formation of sucrose or polysaccharides. Scheme 3 represents the carbon cycle and its relation to the EM pathway in a sim-
1. IODOACETATE AND IODOACETAMIDE

Figure 1: Photosynthetic carbon cycle and its relation to the EM pathway.

Simplified form, the condensation of CO$_2$ with ribulose-1,5-diP forming 3-P-glycerate via a keto acid intermediate. Both Mortimer (1960) and Kandler et al. (1961) have presented evidence that this is not the whole story. First the 3-PGDH of plants has been shown to be less sensitive to iodoacetate than the mammalian enzyme (page 19), being inhibited significantly only above 0.1 mM, whereas photosynthesis is affected strongly at much lower concentrations. Second, the changes in the patterns of C$^{14}$ incorporation do not entirely correspond to a block solely at 3-PGDH. For these reasons another pathway whereby the keto acid can form fructose-1,6-diP more
directly was suggested; this is indicated by the dashed arrow in Scheme 3. It is believed, at least in certain plants, that this is the normal pathway taken in the synthesis of sucrose or polysaccharide, and that this pathway is the most sensitive to iodoacetate, inhibition occurring between 0.001 and 0.01 mM. The various possible sites of inhibition, as given by Kandler et al. (1961), are indicated in Scheme 3 by the pI₅₀ values. At low concentrations only the keto acid → fructose-1,6-diP reaction is blocked, but at higher concentrations the inhibition may be more complex and involve at least four sites.

It has been thought that inhibition of CO₂ incorporation might be due to an inhibition of the formation of ribulose-1,5-diP, with which CO₂ condenses, particularly by a block of the ribulose-5-P kinase. However, although this enzyme is inhibited by iodoacetate, it is not as sensitive as total photosynthesis, and one must attribute a minor role to this site of inhibition. Of course, formation of ribulose-1,5-diP may be decreased if any step in the carbon cycle is inhibited, and this probably occurs if the site is the keto acid → fructose-1,6-diP reaction. Furthermore, there is no accumulation of ribose-5-P in iodoacetamide-poisoned plants (Gibbs and Calo, 1960 a). The ribulose-1,5-diP carboxylase is unaffected by 10 mM iodoacetamide (Gibbs and Calo, 1959 a) so this cannot be the site of inhibition. One is left with only the keto acid → fructose-1,6-diP reaction as the sensitive step, but no direct studies of this have been reported.

The question of 3-P-glycerate levels during inhibition of photosynthesis has been much discussed, and some have assumed that this substance should accumulate, particularly if 3-PGDH is the site of inhibition. Change in concentration of intermediates in such complex systems is, however, often difficult to predict (see page I-514). Here we have a cycle with pathways issuing from some of the cycle intermediates, even neglecting for the moment the postulated shunt across the carbon cycle. There is no necessity at all for 3-P-glycerate to accumulate during iodoacetate inhibition; it may or it may not. First, if its utilization along the EM pathway is decreased, more will presumably proceed along other pathways, e.g., the formation of serine-P or pyruvate (and other amino acids derived from the tricarboxylic cycle). This has actually, been observed and is undoubtedly the explanation for the rise in C¹⁴ incorporation into amino acids and malate. Second, since CO₂ incorporation is inhibited, less 3-P-glycerate would be formed (assuming only the Calvin cycle); accumulation of a cycle intermediate is usually very moderate when a cycle step is inhibited. If one now introduces the keto acid → fructose-1,6-diP shunt, a further consideration rises. If this is the normal pathway for the keto acid, a block of this reaction will increase the hydrolysis of the keto acid to 3-P-glycerate, and in this way the level of 3-P-glycerate might tend to rise, particularly at low iodoacetate concentrations. According to this picture, iodoacetate at low concentrations
forces photosynthesis into the Calvin carbon cycle. This could also contribute to the greater formation of malate and amino acids.

The fixation of $^{13}$CO$_2$ by broken spinach chloroplasts in the presence of either ribose-5-P or ribulose-1,5-diP and the effects of iodoacetamide on this were studied by Trebst et al. (1960). It was stated that the carboxylative phase is strongly inhibited but not the reductive phase. The accompanying tabulation of the results shows that the fixation with ribose-5-P as sub-

<table>
<thead>
<tr>
<th>Iodoacetamide (mM)</th>
<th>% Inhibition of $^{13}$CO$_2$ fixation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ribose-5-P</td>
</tr>
<tr>
<td>0.1</td>
<td>13</td>
</tr>
<tr>
<td>1</td>
<td>70</td>
</tr>
<tr>
<td>10</td>
<td>96</td>
</tr>
</tbody>
</table>

strate is inhibited somewhat more than with ribulose-1,5-diP, which indicates some inhibition, not of the carboxylative phase but of the formation of ribulose-1,5-diP, an inhibition which could be on either pentose-P isomerase or ribulose-5-P kinase, probably the latter. On the other hand, the carboxylation of ribulose-1,5-diP is only weakly inhibited. Indeed, neither of these inhibitions is very potent and could not explain the depression of photosynthesis at concentrations below 0.1 mM.

One must be careful in making generalizations about the mechanism of iodoacetate inhibition, inasmuch as all plants do not photosynthesize in exactly the same way. Mortimer (1960) found sugar beet and soybean leaves to have somewhat different photosynthetic systems, the former depending partially on 3-PGDH and the latter not at all; and Kandler et al. (1961) reported quite different behavior in two species of Chlorella. Since only a few species of plants have been studied thoroughly, it is very probable that different patterns of response to iodoacetate will be found, and that the above conclusions will not be generally valid.

**Nonphotosynthetic (Dark) CO$_2$ Fixation**

Studies of photosynthesis have generally not included measurement of CO$_2$ fixed in the dark. Since the rate of dark fixation is probably much lower than in the light, it is likely that it does not significantly complicate the photosynthetic incorporation. However, the nonphotosynthetic fixation of CO$_2$ may be quite sensitive to iodoacetate. Wood and Werkman (1940) found that CO$_2$ fixation in Propionibacterium pentosaceum (whereby succinate is formed from glycerol and CO$_2$) is inhibited 69% by 0.078 mM iodoacetate. This is presumably not due to a direct inhibition on the car-
boxylation, for the new type of CO$_2$ fixation reaction found in propionic acid bacteria:

$$\text{Phosphoenolpyruvate} + \text{CO}_2 + P_i \leftrightarrow \text{oxalacetate} + \text{PP}$$

is not depressed by iodoacetamide up to 4 mM (Siu and Wood, 1962). The fixation of C$^{14}$O$_2$ by yeast endogenously is inhibited 35% by 0.046 mM iodoacetate, and that induced by glucose is completely blocked at this concentration (Stoppani et al., 1958 a). The action may not be on CO$_2$ fixation itself but on the formation of phosphoenolpyruvate, or other substances, for condensation of the CO$_2$. The usual carboxylation of phosphoenolpyruvate in plants is not affected by even 10 mM iodoacetamide (Tchen and Vennesland, 1955).

**SPECIFICITY OF INHIBITION OF 3-PGDH AND EM PATHWAY**

There is no doubt that a large number of enzymes and metabolic pathways are appreciably inhibited by iodoacetate. The fundamental problem in a particular instance is whether a concentration specifically blocking the EM pathway can be found. The answer will depend on several factors — the relative susceptibilities of the enzymes involved, the permeability of the cells to iodoacetate, the pattern of metabolic activity under the experimental conditions, the duration of contact with the inhibitor — and, in addition, on what cellular process is being measured. It appears to be possible to produce a specific inhibition of 3-PGDH within the EM pathway itself by using concentrations in the range 0.05–0.2 mM, although 3-PGDH may not be completely blocked in all cases. It is also likely that the EM pathway can be appreciably inhibited without interfering significantly with the oxidation of pyruvate or the operation of the cycle, but this is difficult and care must be taken in choosing the experimental conditions. In much work attempting to correlate cellular activities with the EM pathway it is quite certain that the cycle was inhibited to varying degrees.

It is fairly difficult to test for the specificity of action on 3-PGDH by determining the accumulation of intermediates since the situation is frequently quite complex, as previously discussed, but it is occasionally possible to differentiate the effects on the EM pathway and the cycle by showing a response to added pyruvate. We shall have occasion to note the results of such addition in studies of cell function, but unfortunately such tests have seldom been done. Certain aspects of this problem have been treated (page 1-877). One very crucial problem here is the permeability of the cells under consideration to pyruvate, since the effect of pyruvate may be limited by its rate of penetration. Another point to be taken into account is the fact that pyruvate cannot completely restore normal condi-
tions in iodoacetate-poisoned cells, because the ATP generated in the EM pathway remains depressed; if compartmentalization of ATP occurs, that produced in the cycle may not be able to replace that from the EM pathway. Numerous examples of failure of pyruvate to counteract effects of iodoacetate have been reported; e.g., the depression of the Na\(^+\) pump and the respiration in the marine alga *Hormosira banksii* by 0.1 mM iodoacetate is not prevented by pyruvate (Bergquist, 1958). In this case a pH of 4.8 was used to facilitate penetration (there was no effect at pH 7.5) and hence one must consider the possibility of an acidification of the cell due to penetration of iodoacetic acid. Pyruvate will not entirely prevent the iodoacetate depression of cardiac tissue; indeed, it will not completely counteract the effects of 2-deoxyglucose, which is certainly specific for the EM pathway. In these cases one can adduce compartmentalization or inadequate penetration, but there is no evidence either way.

The specificity question becomes especially acute in long-term experiments where many metabolic pathways are involved, as in the inhibition of synthesis and growth. Many of the enzymes necessary for the over-all function, maintenance, and growth of cells have not yet been examined with respect to inhibition by iodoacetate. The time relations may be critical. Selective attack on 3-PGDH or the EM pathway is often possible because 3-PGDH generally reacts more rapidly than other enzymes with iodoacetate, so that over a period of 30-60 min some specificity may be achieved, but the longer the contact with iodoacetate, the greater the likelihood of other loci of action. Many of the results obtained with iodoacetate are meaningless and have led to erroneous interpretations because the factor of time has been neglected.

**PENETRATION INTO CELLS AND THE EFFECTS OF pH**

Iodoacetic acid is a weak acid (pK\(_a\) = 3.12) and in the physiological pH range would exist primarily in the ionized form of iodoacetate. Cells with permeability barriers to anions should thus be relatively resistant to this inhibitor, the effects increasing as the pH is reduced. The pH dependence of iodoacetate inhibition was first noted in yeast fermentation by Neuberg and Kobel (1931), who stated, "*Das jodessigsaur*e Salz bei Verschiebung des pH nach alkalischen Seite etwas in seiner Wirkung behindert ist."* and the quantitative aspects of fermentative and respiratory inhibition were studied by Lundsgaard (1932). Many workers since then have reported essentially complete inhibitions at pH 4.0-4.5 whereas little or no inhibition occurs if the pH is raised above 7. Such is seen in yeast fermentation (Brücke, 1933;

* "The action of the iodoacetic acid salt is reduced by a displacement of the pH in the alkaline direction."
Ehrenfest, 1932), yeast respiration (Beeveres and Simon, 1949), yeast growth (Schroeder et al., 1933 a; Rapkine, 1937; Aldous, 1948), Torula growth (Hansen, 1956 a), algal respiration and transport (Bergquist, 1958). Chlorella photosynthesis (Fraser, 1954), Avena coleoptile growth (Cooil, 1952), and barley root respiration (Laties, 1949 a). A typical inhibition-pH curve for yeast fermentation is given in Fig. I-14-21. Since the pH affects the rate of penetration of the inhibitor and the intracellular concentration, inhibition will occur at the higher pH’s if sufficient time is allowed, as shown in Fig. I-14-20. In the examples above, the inhibition is roughly proportional to the concentration of undissociated iodoacetic acid and the same relationship can be obtained by varying the total concentration at a constant pH (Aldous, 1948). However, Simon-Beevers curves for the effect on yeast respiration (Fig. I-14-23) show that the situation is not quite this simple, the curve for the undissociated acid not being horizontal, indicating either a penetration or membrane effect by the iodoacetate anion, or a reduction in the inhibition resulting from a fall in the intracellular pH at low external pH’s. Although little work has been done with iodoacetamide with respect to pH, it is worth noting that Kohn (1935) found a decrease in the pH from 9.2 to 5.3 to increase the inhibition of photosynthesis by iodoacetamide, an effect which is difficult to explain unless one assumes some change in the permeability properties of Chlorella. Failure of iodoacetate to inhibit can often be attributed to lack of penetration at an unfavorable pH. For example, Lewin and Mintz (1955) reported that respiration and photosynthesis are scarcely affected by 50m M iodoacetate in Chlamydomonas, but the pH was 8.5 and the inhibitor was added from the side-arm of the vessels, so that probably iodoacetate simply did not get into the cells.

The marked pH effects discussed above were all observed in yeast and plant cells. Quite different results have been obtained in the few studies on mammalian tissues. The hemolysis of erythrocytes by iodoacetate was related to an inhibition of glycolysis by Wilbrandt (1937), but the effect is weakened as the pH is decreased from 7.4 and is abolished at pH 5.9. This indicates an action on the membrane rather than one requiring penetration into the cells. The respiration of rat brain suspensions is inhibited by iodoacetate and this is independent of pH from 6.7 to 7.8 (Bernheim and Bernheim, 1941), but there is a question whether this was a cellular preparation. Certainly many mammalian tissues are metabolically and functionally depressed by iodoacetate at physiological pH, but the concentrations necessary for inhibition are often rather high (1-10 m M) and this may indicate that penetration is poor. If one assumes that iodoacetate at 0.01-0.1 m M inhibits the EM pathway, it is reasonable to estimate that around 100 times these concentrations must be present in the extracellular medium. With respect to the concentration of undissociated iodoacetic acid, increasing the total concentration 100 times is essentially the same as keeping the total
concentration constant and reducing the pH by 2 units. Although plant cells may generally be less anion-permeable than mammalian cells, there is no good evidence to indicate a marked difference in the effects of pH on iodoacetate inhibition.

Iodoacetamide was introduced to facilitate penetration, and little effect of pH on the entrance of this substance into cells would be anticipated. This was confirmed by Stannard (1938 a) for frog muscle, iodoacetamide inhibiting respiration more rapidly than iodoacetate at physiological pH whereas in acid media the two inhibitors are equally effective. However, there are very good reasons for some effect of pH on the actions of iodoacetamide. First, the permeabilities of cells are modified by pH and, second, the intracellular pH and hence the rate of iodoacetamide reaction with SH groups may be changed by the change in external pH.

A nonspecific intracellular acidification due to the penetration of undissociated iodoacetic acid at low pH's has seldom been considered as playing a role in the effects observed. The concentrations of iodoacetic acid at different pH's and at a total concentration of 1 mM are shown in the accompanying tabulation. Iodoacetic acid will enter the cells and dissociate into

<table>
<thead>
<tr>
<th>pH</th>
<th>Iodoacetic acid (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>$1.32 \times 10^{-7}$</td>
</tr>
<tr>
<td>6</td>
<td>$1.32 \times 10^{-6}$</td>
</tr>
<tr>
<td>5</td>
<td>$1.30 \times 10^{-5}$</td>
</tr>
<tr>
<td>4</td>
<td>$1.16 \times 10^{-4}$</td>
</tr>
<tr>
<td>3</td>
<td>$5.70 \times 10^{-4}$</td>
</tr>
</tbody>
</table>

iodoacetate and H+. As the iodoacetate reacts with cellular components, more iodoacetic acid will enter until at equilibrium the concentrations inside and outside are the same. In addition, H+ may be released during the carboxymethylation of SH groups. The effect on intracellular pH will depend on the buffering capacity of the cell, and at external pH above 6 there is likely to be little change in intracellular pH. However, at lower pH's substantial decreases in intracellular pH are very likely to occur, when one considers the amounts of iodoacetic acid entering the cells and the final equilibrium concentrations. Such intracellular pH alterations may have two important effects: (1) a direct effect on metabolic or functional processes, and (2) a reduction in the rate of inhibition by iodoacetate, since acidification slows the reaction of iodoacetate with SH groups. The cell pH may also be modified by the metabolic disturbance produced by iodoacetate (see page 82). The marked inhibitions of several intracellular yeast en-
zymes by iodoacetate at 1.5 mM and a pH of 4.5 observed by Aldous (1952) may be explained by a nonspecific acid effect, especially since some of these enzymes are not readily inhibited by iodoacetate.

**EFFECTS ON PERMEABILITY AND ACTIVE TRANSPORT**

Cellular function usually depends in one way or another on the transport of substances into or out of the cell, and excitable tissues apparently have specialized systems for membrane transport. It will therefore be necessary to discuss the inhibition of these general processes before taking up individual cellular activities. Inasmuch as membrane transport inhibition was treated very cursorily in Volume I (page 439), the opportunity will now be taken to discuss some general aspects of this problem, especially as it relates to the effects of SH reagents and other irreversible or slowly irreversible inhibitors.

**Mechanisms of Inhibition of Transport and Accumulation**

The transport of a substance through the plasma membrane or across a layer of cells may be *passive* (simple diffusion), *facilitated* (diffusion of a complex with a carrier), or *active* (requiring energy from metabolism). In all cases the inhibitor can produce its effect by acting either on the metabolic systems involved in supplying energy for transport or the maintenance of the membrane, or on the membrane or the transport system directly. For example, the permeability to a substance can be altered by an attack on the membrane and its structure, or secondarily by disturbing metabolism. Some inhibitors, such as cyanide or fluoroacetate, undoubtedly act mainly by the latter mechanism, whereas SH reagents are much more apt to act also on the membrane, since the membrane proteins contain SH groups probably important in maintaining the integrity of the membrane. Active transport systems may for convenience be considered as consisting of two parts: the *exergonic reactions* providing the required energy (usually oxidations coupled with ATP generation) and the *endergonic reactions* by which the substance is transported and the energy utilized (the functional transport system itself). Inhibitors are often used to detect active transport and are assumed to reduce the supply of energy, but many inhibitors can also affect the transport system directly, i.e., either the exergonic or endergonic phase may be depressed. Inhibitors reducing ATP generation (e.g., 2,4-dinitrophenol) can be thought of as exerting a rather nonspecific effect with respect to transport, since any cell function requiring energy will be depressed eventually, whereas inhibition of the endergonic system may be more selective, blocking transport and leaving other cell functions intact (except as they may be secondarily modified), or even blocking one trans-
port system of several.* Wilbrandt has termed primary inhibitors those which act directly on transport systems and secondary inhibitors those depressing metabolic energy formation.

One should perhaps avoid the assumption that there is a general energy production intracellularly, the ATP formed being used by all the functional processes, e.g., that in muscle the ATP generated in the EM pathway and the cycle is used indiscriminately for maintenance of the membrane potential and for contraction. There is some evidence that compartmentalization of ATP occurs occasionally and that the ATP used in a particular process may be generated in the region of that process. It is possible that the EM pathway and the cycle provide energy in a definite pattern, and that exergonic sequences occur in the membrane and are especially involved in active transport.

Factors Involved in the Intracellular Accumulation of Substances

Although it is not necessary that active transport lead to a higher concentration of a substance within the cell than in the medium, this is the usual result and often one criterion for active transport. The effects of an inhibitor on the accumulation of a substance are frequently determined and the results interpreted in terms of modification of active transport. Iodoacetate generally reduces the accumulation, whether transmembrane or transcellular, and often induces a loss of the substance from the cell, as is evident from the summary presented in Table 1-31. Thus iodoacetate not only depresses the accumulation of K+ in K+-depleted cells but causes a loss of K+ from normal or semidepleted cells. The interpretation of such data is more complex than in simple transport systems. There are two opposing processes: the active transport of the substance in one direction, and the passive diffusion of the substance in the other direction, the latter being determined by the permeability of the membrane for the substance. The higher the permeability, the greater the rate of diffusion and the more active the transport mechanism must be to lead to accumulation or maintain a high intracellular level of the substance. If an inhibitor is found to reduce accumulation or lead to loss of a substance from the cell, it could be acting by two general mechanisms: depression of the active transport or acceleration of the rate of loss. The latter will be brought about by an increase in membrane permeability and such an action is more likely for those inhibitors reacting with protein groups.

* The division of possible sites of action into exergonic and endergonic does not imply that only the former may be enzymic. Enzymes are undoubtedly often involved in the transport system in the membrane; one example is ATPase. Effects on the transport system can be through several different mechanisms and are not to be interpreted only as structural changes.
At equilibrium the steady state may be represented by:

\[ v_{ae} = v_{de} = P \Delta C_e \]  

(1-1)

where \( v_{ae} \) is the equilibrium active transport rate, \( v_{de} \) is the equilibrium outward diffusion rate, \( P \) is a permeability constant, and \( \Delta C_e \) is the concentration difference between the inside and the outside of the cell. During active uptake of the substance the rate of accumulation (\( v_c \)) is given by:

\[ v_c = v_a - v_d = Kf(\Delta C) - P\Delta C \]  

(1-2)

where \( v_a \) and \( v_d \) are the active inward and passive outward rates, respectively, \( K \) is a constant characterizing the active transport system, \( \Delta C \) is the concentration gradient, and \( f(\Delta C) \) represents some function of the concentration gradient. As accumulation proceeds, \( v_c \) decreases and eventually comes to zero at equilibrium; this results certainly from an increase in \( v_d \) and probably also from a decrease in \( v_a \). An inhibitor may reduce accumulation by either decreasing \( K \) or increasing \( P \), i.e., slowing active transport or increasing the permeability.

In order to determine the behavior of such a system we must assume some relationship between active transport and \( \Delta C \). It is likely that the rate of transport declines as the accumulation proceeds and is minimal at equilibrium, inasmuch as oxidative metabolism in a tissue such as nerve is usually low in a resting state but increases if a series of impulses alters the gradients of Na\(^+\) and K\(^+\). The simplest and most logical function would be \( v_a = K(\Delta C) \), the rate of transport slowing as \( \Delta C \) increases and stopping when it reaches some value designated as \( A \), so we may now write:

\[ v_c = \frac{d(C_i)}{dt} = K(A - \Delta C) - P\Delta C \]  

(1-3)

which may be integrated to:

\[ (C_i) = \left[ \frac{KA}{K + P} + (C_o) \right] \left[ 1 - e^{-\left(\frac{K}{K + P} \right)t} \right] \]  

(1-4)

where \((C_i)\) and \((C_o)\) are the concentrations inside the cell and in the medium, respectively. As accumulation proceeds and \( t \) becomes larger, the equilibrium state is eventually reached, at which time the concentration gradient is

\[ \Delta C_e = \frac{K A}{K + P} \]  

(1-5)

The rate of accumulation and the equilibrium gradient thus depend on the relative values of \( K \) and \( P \), and hence the effect of an inhibitor, acting on either active transport or the permeability, will be determined by both.
### Table 1-31
**Effects of Iodoacetate on Active Transport Processes**

<table>
<thead>
<tr>
<th>Transported substance</th>
<th>Organism and tissue</th>
<th>Iodoacetate (mM)</th>
<th>% Inhibition</th>
<th>General effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inorganic cations</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na⁺—K⁺</td>
<td><em>Escherichia coli</em></td>
<td>0.9</td>
<td>50</td>
<td>—</td>
<td>Roberts <em>et al.</em> (1949)</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td>1</td>
<td>&gt;95</td>
<td>—</td>
<td>Schultz and Solomon (1961)</td>
</tr>
<tr>
<td></td>
<td>Yeast</td>
<td>0.1</td>
<td>Inh.</td>
<td>—</td>
<td>Pulver and Verzár (1940)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>≈100</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yeast</td>
<td>0.1</td>
<td>—</td>
<td>(K⁺)ᵢ &lt; 20%</td>
<td>Scott <em>et al.</em> (1951)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>—</td>
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References:
- Huf et al. (1957)
- Armstrong (1964)
- Zadunaisky and Curran (1963)
- Wickson-Ginzburg and Solomon (1963)
- Elsbach and Schwartz (1959)
- Craig (1959)
- Whittam and Breuer (1959)
- Kinoshita et al. (1961)
- Curran (1960)
- Mudge (1951)
- Kean et al. (1961)
- Keynes and Maisel (1954)
- Manery et al. (1956)
- Van der Kloot (1958)
- Muller (1962)
- Del Monte (1961)
- Shanes (1952)
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**Inorganic anions**

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**Carbohydrates**

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**References**

- Machlis (1944)
- Ordin and Jacobsen (1955)
- Acland (1962)
- Becker (1961)
- Welch (1962)
- Slingerland (1955)
- Stickland (1956c)
- Kanam and Spiegelman (1948)
- Hoffmann-Ostenhof and Kriz (1959)
- Arisz (1959)
- Rönnin (1953)
- Schoffeniels (1951)
- Müller and Kliefield (1953)
- Ord and Stoecken (1961)
- Bieleni (1966)
- Cardisky and Huang (1962)
- Wilbrandt and Lasz (1944)
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</tr>
<tr>
<td></td>
<td>Rat intestine</td>
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<td>80</td>
<td>Hackett and Thimann (1950)</td>
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<td>Nevis (1958)</td>
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<tr>
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<td>Rat intestine</td>
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<td>Smyth and Taylor (1955)</td>
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</tr>
</tbody>
</table>

* Key to symbols: \( (X)_i \) = concentration of substance inside cells; \( \Delta (X)_i \) = change in intracellular concentration during incubation; \( K/Na \) = ratio of intracellular concentrations of \( K^+ \) and \( Na^+ \); \( T/M \) = ratio of concentrations in tissue and medium; IAM = iodoacetamide; \( BrA \) = bromoacetate.

* These values corrected for simple diffusion rates.
A simple calculation will illustrate this. If we assume different initial values of $K/P$ and that the inhibitor reduces $K$ by 50%, the depressions of $\Delta C_e$ will vary as shown in the accompanying tabulation. The effect of the inhibitor on $\Delta C_e$ will be greater the lower the $K/P$ ratio. This is on a per cent basis; the absolute loss of the substance from the cells will be greater in the midrange of $K/P$. If one starts out with depleted cells, the inhibition of the accumulation rate is given by:

$$
\frac{\Delta C_e - \Delta C}{\Delta C_e - \Delta C} \frac{K}{K + P} \frac{i}{i_e}
$$

where $i_e$ is the inhibition of active transport. If $P$ is very small relative to $K$, the inhibition of the accumulation rate will be essentially the same as the inhibition of active transport, but if $P$ is larger the inhibition of the accumulation rate will be reduced. These simple examples are presented to demonstrate that the inhibition experimentally observed, either on $\Delta C_e$ or $v_e$, is not necessarily the same as on the active transport process, and that changes in the permeability can bring about the same effects as changes in transport. In the system assumed here it is also clear that an inhibitor could reduce $\Delta C_e$ to a lower but stable value; with iodoacetate this is not so likely since the inhibition increases progressively with time and the gradient will likewise steadily fall.

A further factor must be considered if the substance transported can be metabolized in the cells, in which case at equilibrium we have:

$$
v_{ae} = v_{ae} + v_{me}
$$

where $v_{ae}$ is the rate of metabolic disappearance at equilibrium. An inhibitor not only can act on transport or diffusion, but also can modify the rate of utilization and thereby affect accumulation as measured by the intracellular concentration of the substance. This factor cannot be neglected in studies with glucose or amino acids, since iodoacetate can affect the utilization of both. Thus rat diaphragm takes up $\alpha$-aminoisobutyrate and leucine aerobically, and iodoacetate at 0.5 mM increases the tissue/medium ratio from 1.09 to 2.68 (Peckham and Knobil, 1962). Even anaerobically
iodoacetate brings about an increase in the ratio from 0.60 to 0.67. This might be due to an inhibition of the utilization of the amino acids since it was shown that incorporation into proteins is reduced, over the experimental period the utilization being depressed more than the uptake.

Equilibrium and Nonequilibrium States

In studying the effects of inhibitors on transport and accumulation, usually two types of experiment are performed. The cells in an equilibrium state with a fully developed concentration gradient of the substance are treated with the inhibitor, and the loss of the substance from the cells is determined. The other type of experiment involves depleting the cells of the substance by incubation in abnormal media or storing at low temperature, and then measuring the reaccumulation of the substance when the cells are restored to normal conditions. From what has been said above it is clear that the results may be somewhat different in each case. In the first type of experiment, the rate of loss will depend not only on how much the pump is inhibited but perhaps even more on the permeability of the membrane to the substance; it is obvious that if the permeability is zero, there will be no loss even though the pump is completely stopped. In the second type of experiment, the inhibition of the accumulation may depend on the permeability, but to a lesser degree, and some inhibition will always be seen. The second method is more sensitive and useful than the former, but is often less physiological in that when an inhibitor is applied to normal tissue it is presumably initially in an equilibrium state.

Another factor upon which the response of the equilibrium state depends is the degree of cellular activity, particularly when one is concerned with ions. For example, a resting nerve with high internal K$^+$ and low internal Na$^+$, and with relatively low permeabilities for these ions, may retain the normal ionic distribution and membrane potential for long periods even though the active transport is blocked. However, if the nerve is stimulated and suffers a series of depolarizations, each resulting in a small loss of K$^+$ and gain of Na$^+$, the concentration gradients will be reduced and the potential will fall. This is one important basis for the relationship between degree of inhibition and functional activity of the tissue.

A final factor is the rate at which the active transport system is operating. In a resting cell at equilibrium the pumps may be working at a low level, whereas in states deviating from equilibrium the pumps will be operating more actively. It is quite possible that one type of exergonic metabolism may be sufficient for the resting state, but that other metabolic energy sources must be tapped when the pumps are very active. Thus the response to an inhibitor may depend on the degree of deviation from an equilibrium condition, not only because of different rates of exergonic metabolism but because of possibly different pathways involved.
Effects of Iodoacetate on Cell Membranes and Permeability

Before discussing the effects of iodoacetate on active transport processes, we shall examine the rather meager evidence that actions directly on the membrane can be exerted. The permeability of erythrocytes to glycerol and various monosaccharides appears not to be affected by even high concentrations of iodoacetate (Wilbrandt, 1937, 1938; Hunter, 1947; Danielli, 1954) the integrity of the membrane here not being dependent on glycolysis.* However, the results with ions are quite different. Iodoacetate causes a loss of K+ from erythrocytes and is lytic (Ponder, 1949; Flynn and Maizels, 1949). It is quite certain that part of the K+ loss is due to structural changes in the membrane. Green and Parpart (1953) believed that the primary action of iodoacetate is on the membrane to facilitate loss of K+, and Love et al. (1955) reported that iodoacetate produces both an inhibition of K+ influx and an increase in efflux. Iodoacetate at 0.125 mM has no effect on erythrocyte K+ but if 5 mM ferricyanide, which has no effect alone, is also present there is a marked loss of K+, and it was concluded that the effects of iodoacetate on permeability are unrelated to glycolysis (Passow and Gruner, 1963). Iodoacetate at 0.1 mM slightly decreases both K+ influx and efflux in the rat uterus but the former more, so there is a net depression of uptake (Daniel, 1963). The inhibition of glucose transport in the intestine was claimed to occur at concentrations of iodoacetate without effect on glycolysis, and the action was attributed to an effect on the membrane or on ATPase (Rummel et al., 1958). The extrusion of Na+ from muscles previously treated to gain Na+ is inhibited by iodoacetate and the Na+ influx actually rises (Frazier and Keynes, 1959). An analysis of the results led to the conclusion that there is an increased permeability to Na+, that the effect is not entirely on glycolysis, and that iodoacetate may react with SH groups in the membrane. These few reports do not invalidate the concept that the primary effect of iodoacetate usually is to inhibit active transport, but indicate clearly that other factors may be involved, and that in the future such studies should include, where possible, data on permeability and evidence for or against membrane structural changes and lysis.

Methods for Localizing the Site of Action of Inhibitors Reducing Accumulation

If accumulation of a substance is inhibited, there are three general sites for the inhibition: (1) the active transport, (2) the membrane permeability, and (3) the metabolic utilization of the substance, if this occurs. The last mechanism is not very common and is fairly easily recognized, so we shall

* It seems strange that the transport of glucose is unaffected by iodoacetate in view of the results with ions and the fact that it is not a simple diffusion. Perhaps more quantitative and reliable methods would demonstrate definite changes.
confine our attention to distinguishing between the first two possibilities. The following methods may be suggested, although in certain instances other approaches may be applicable. The use of radioactively labeled substances, especially in studies of ions, can provide information on influx and efflux rates independently of total or over-all changes, but a good deal of caution is necessary in their interpretation. It is best if such studies can be done under a variety of conditions. For example, if the active transport is purely aerobic, anoxia will abolish it; the rate of efflux under these conditions should be compared with that in the presence of iodoacetate. If the rate of loss is much greater with iodoacetate, one can conclude that some membrane action has been exerted. It is also occasionally possible to compare the effects of the inhibitor on the accumulation of different substances which are chemically similar, some of these preferably not being actively transported, since a change in permeability will generally be reflected in the movements of all these substances, whereas an inhibition of active transport will affect some more than others. The variation of the inhibition with pH may provide useful information; if the site of action is within the cell, the inhibition will be greater at lower pH's where penetration is better, but if it is on the membrane the inhibition may be greater at higher pH's. However, one is not certain that the pertinent metabolic systems are within the cell, and the surface effects could be on the transport systems themselves.

If the action is shown to be on active transport, the site of inhibition may be on either the exergonic or endergonic phase. The simplest and most used method for distinguishing these is to attempt to establish whether there is a correlation between metabolic and transport inhibitions. In the case of iodoacetate, inhibition of glycolysis and transport may be simultaneously determined, but since there is no necessary quantitative relationship here, as previously discussed, this is usually very unsubstantial evidence. Overcoming of the inhibition by pyruvate is better evidence for a block of the EM pathway. Such correlations will be discussed in the following sections; only under exceptional conditions do they provide valid proof for a site of action. In this connection, analyses for intracellular ATP and creatine-P can be of some value. A fall in these high-energy substances implies an inhibition of exergonic reactions, while a lack of change or an increase suggests an action on the transport systems themselves.

Two particularly ingenious and useful localization techniques were proposed by Davenport et al. (1955) in connection with their work on the inhibition of gastric secretion. The first procedure involves the determination of the inhibitions of both exergonic and endergonic processes (e.g., lactate formation and HCl secretion) under a variety of conditions. If there is a consistent correlation between these inhibitions under all conditions, it is likely that the action is on the exergonic phase, whereas if the correlation
is variable a direct action on transport mechanisms is more likely. One difficulty in using this technique is that the appropriate conditions must be selected, since otherwise a consistent correlation may be merely fortuitous. Actually, the inhibitions of H+ transport and of lactate formation from glucose in gastric mucosa by iodoacetamide show a fairly good and consistent correlation under different conditions; this leads to the conclusion that the EM pathway is the primary site of action. However, when acetoacetate is used as the substrate, little lactate is formed and this is not affected by iodoacetamide, although the inhibition of H+ transport is identical to that when glucose is the substrate. These results might be interpreted in different ways. The EM pathway could still be operative in the presence of acetoacetate and necessary for transport, or the iodoacetamide could be blocking pyruvate utilization, or it could be acting on the transport system itself.

The second procedure involves a determination of the effect of the inhibitor on the inhibition-concentration curve for 2,4-dinitrophenol and is perhaps more reliable than the previous method. It was described briefly on page I-504 and illustrated in Fig. I-10-6. One assumes that 2,4-dinitrophenol depresses cell function by uncoupling oxidative phosphorylation and reducing the supply of ATP. If the inhibitor being studied also inhibits ATP formation, it should require less 2,4-dinitrophenol to initiate inhibition of function, whereas if the inhibitor acts on the functional system, more 2,4-dinitrophenol may be required. Iodoacetamide shifts the inflection point of the 2,4-dinitrophenol curve to the right, i.e., to higher concentrations of the uncoupler, and thus appears to exert its primary action on the endergonic or transport phase. Such an approach could be profitably combined with analyses for ATP.

When the transport of a particular substance is found to be inhibited, care must be exercised in immediately attributing the site of the action to a system responsible for the movement of this substance specifically. The transport of one ion must involve the movement of another ion to maintain electrical neutrality. In many situations the transports of Na+ and K+ are linked and it is very difficult to determine whether one or the other or both are actively transported, and similarly in the kidney there has been prolonged argument as to the primary ion resorbed in the proximal tubules (Na+ or Cl–). The transport of water in many tissues (e.g., the kidney or the ciliary body of the eye) is for the most part secondary to ion transport. If an inhibitor causes a cell to swell, one is not justified in locating the action on a water-transport system, nor in concluding that normally water is actively pumped out of the cell, since the water may merely follow changes in ion distribution. There are examples where Na+ and K+ transports seem to be linked and yet a certain inhibitor will exhibit a selective effect. The Na+ extrusion and K+ uptake in E. coli are affected similarly by iodo-
acetate and 2,4-dinitrophenol, but floride can inhibit Na⁺ transport completely while having only a minor effect on K⁺ uptake (Schultz and Solomon, 1961).

**Correlations between Transport Inhibition and Depression of Glycolysis**

Many workers have claimed to have established the basis for transport inhibition by iodoacetate as the EM pathway — e.g., in yeast (Pulver and Verzár, 1940), duck erythrocytes (Tosteson and Johnson, 1957), human erythrocytes (Wilbrandt, 1940), toad muscle (Muller, 1962), Ehrlich ascites cells (Maizels et al., 1958), calf lens (Kinoshita et al., 1961), rat diaphragm (Del Monte, 1961), and nerve (Shanes, 1952), but in most instances the only evidence has been that a depression of glucose uptake, lactate formation, or CO₂ release occurs simultaneously with the inhibition of the transport. There is seldom quantitative correlation between the inhibitions and none would be expected: sometimes glycolysis is reduced much more than transport — as in ascites cells, where glycolysis is inhibited 60% and Na⁺ efflux 36% by 0.07 mM iodoacetate, and in duck erythrocytes, where glycolysis is inhibited 96% and K⁺ influx 50% by 1 mM iodoacetate; but in other cases it seems that rather slight glycolytic inhibition causes marked changes in transport — as in calf lens, where a 31% depression of glycolysis is accompanied by a marked block of K⁺ accumulation, or in toad muscle, where a 34% inhibition of glycolysis is again accompanied by severe loss of K⁺ and gain of Na⁺. Thus these data alone do not signify the mechanism of the inhibition at all. A good example of this is given by Craig (1959), who found that perfused frog liver loses K⁺ as lactate formation is inhibited by iodoacetate, and indeed the concentrations necessary to produce comparable inhibitions are roughly the same; one might conclude that the accumulation of K⁺ is dependent on glycolysis. However, when the iodoacetate is washed out, the loss of K⁺ stops while the block of glycolysis persists, leading Craig to conclude that K⁺ movements are really not related to lactate formation. In at least one case, rabbit kidney slices, 1 mM iodoacetate has no effect on K⁺ concentrations, K⁺ flux, or the exchangeable and nonexchangeable fractions, so that it was concluded that K⁺ transport here is independent of glycolysis (Mudge, 1953).

Efforts have been made to correlate the effects of iodoacetate on transport and respiration, but it is clear from what has previously been said about respiratory inhibition (page 111) that any relationship observed would be fortuitous. One might predict respiration to be depressed less than transport, and this is borne out in several studies. The following may be cited (in each case the inhibition of transport is given first and then the respiratory inhibition): Br⁻ uptake in barley roots (30%-18% by 0.01 mM iodoacetate, 90%-62% by 0.05 mM iodoacetate) (Machlis, 1944), K⁺ uptake by barley roots (81%-24% by 1 mM iodoacetate) (Ordin and Jacobson, 1955), glycine
uptake by mustard roots (46%-36% by 0.1 mM iodoacetate) (Wright, 1962), NaCl transport in frog skin (48%-0% by 1 mM iodoacetate) (Huf et al., 1957), I- uptake by sheep thyroid slices (93%-34% by 1 mM iodoacetate) (Slingerland, 1955), and K+ uptake in rabbit kidney slices (93%-49% by 0.33 mM iodoacetate) (Mudge, 1951). If respiration were a measure of the rate of ATP generation and if all the ATP formed were available for transport, some correlation might occur, but it is likely that only certain fractions of the respiration are blocked by iodoacetate and that compartmentalization of ATP complicates the picture. The effects of iodoacetate on transport are much more specific than could be demonstrated by overall respiratory measurements.

A little work has been done with iodoacetate on the problem of the dependence of transport on ATP and creatine-P levels, although the results are not as clear as those obtained with uncoupling agents. Ling (1951) showed that the loss of K+ from muscle due to iodoacetate and anoxia can be prevented by cooling to 1°. Since metabolism is essentially stopped at 1° and ATP + creatine-P remain the sole sources of energy, it was felt that K+ accumulation depends on the level of these high-energy substances. A correlation between cell K+ and levels of ATP and creatine-P (especially ATP) was also demonstrated and iodoacetate was assumed to reduce the cell K+ by reducing ATP. This was interpreted in terms of the fixed charge hypothesis but this is not necessary. Whittam (1958) and Whittam and Breuer (1959) have reported evidence that K+ uptake in human erythrocytes and guinea pig seminal vesicle is dependent on the level of ATP, but the evidence is simply that K+ loss is accompanied by a drop in ATP. In calf lens there is certainly very little decrease in ATP when Na+ extrusion is markedly interfered with by 0.03-0.1 mM iodoacetate, and here some more specific action on transport is likely (Kinoshita et al., 1961). The increase in sugar transport in the rat diaphragm brought about by iodoacetate is also not correlated with significant changes in ATP or creatine-P (Kono and Colowick, 1961). One of the few examples in which exogenous ATP affects active transport is the uptake of thiamine by Lactobacillus fermenti. The stimulation of the transport by glucose is inhibited by high concentrations of iodoacetate (20-100 mM) and this is effectively countered by the addition of ATP (Neujiahr, 1963). ATP alone has no effect on thiamine uptake.

Antagonism of Iodoacetate Inhibition by Pyruvate and Other Substrates

If depression of transport by iodoacetate is due to a selective block of the EM pathway, the addition of pyruvate (or some related substance oxidized through the cycle) should overcome the inhibition provided (1) the pyruvate can penetrate satisfactorily into the cells, and (2) the energy generated in the cycle can be used for the transport. Most of the reports have
indicated either partial antagonism or, more usually, none at all. Iodoacetate at 0.2–0.5 mM inhibits the accumulation of phenol red in isolated kidney tubules. Beck and Chambers (1935) found pyruvate to counteract this inhibition partially and lactate to be less effective, but Forster and Taggart (1950) and Jaffee (1954) observed no effect of lactate added before, with, or after iodoacetate (0.5 mM). Similarly Wilbrandt (1940) obtained in erythrocytes partial reversal of the iodoacetate inhibition of the Na+-K+ transport (even though iodoacetate was as high as 10 mM), whereas Maizels (1951) found only very slight antagonism. In algae the situation is more complex. The extrusion of Na+ and accumulation of K+ by Ulva lactuca are inhibited by 1 mM iodoacetate in the dark, the K+ loss being greater than the gain in Na+ (Scott and Hayward, 1954). Pyruvate completely prevents the rise in cell Na+ induced by iodoacetate but only partially antagonizes the K+ loss. In Hormosira banksii 0.1 mM iodoacetate leads to an increase in cell Na+ and a progressive decline in respiration; pyruvate does not modify either action (Bergquist, 1958). Iodoacetate at 1 mM reduces the K+/Na+ ratio in frog muscle from 5 to 3.4; in the presence of pyruvate the ratio is reduced to 3.9 (Van der Kloot, 1958). This rather weak antagonism led Van der Kloot to conclude that the site of action is mainly the EM pathway, and from work with other inhibitors that energy from either the cycle or glycolysis can be used for Na+ extrusion. However, it would be more reasonable to conclude that iodoacetate is at least affecting significantly systems other than the EM pathway. Indeed, all of these studies point to an inability of pyruvate to counteract completely or at all the transport effects of iodoacetate. This should not be interpreted as proving that the principal site of action is not on the EM pathway, but certainly suggests this, especially since penetration of iodoacetate would make likely the penetration of pyruvate and since cycle energy seems to be able to support transport in most instances.

If a substrate stimulates transport in the uninhibited preparation, it is sometimes difficult to determine if antagonism of inhibition occurs. The accumulation of Br− by barley roots is definitely stimulated by various cycle intermediates, in both the absence and presence of iodoacetate (Table 1-32) (Machlis, 1944). The percentage inhibition by iodoacetate is less in the presence of any of these intermediates than in the endogenous control. This could be interpreted as antagonism. However, what is actually shown is that 0.01 mM iodoacetate does not interfere with the transport-promoting activities of these substrates, while 0.05 mM iodoacetate definitely depresses their effects. The data do not prove that the cycle as a whole is unaffected (e.g., pyruvate oxidation might be inhibited) or that the site of action of iodoacetate, even at 0.01 mM, is entirely on the EM pathway. A similar situation is found in the uptake of tyrosine by rat brain, where glucose increases the cell/medium ratio from 2.73 to 4.63 and in the pres-
### Table 1-32a

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Iodoacetate (mM)</th>
<th>Br⁻ Accumulation</th>
<th>Respiration</th>
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<tr>
<td></td>
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<td>% of control</td>
<td>% Inhibition</td>
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<td>—</td>
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<td>62</td>
<td>74</td>
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</table>

*From Machlis (1944).*

ence of 10 mM iodoacetate increases it from 1.69 to 2.57 (Guroff et al., 1961). If anything, this indicates that the primary action of iodoacetate is on the cycle, inasmuch as glucose should not overcome a block of 3-phosphoglyceraldehyde dehydrogenase. On the other hand, possibly glucose plays a role in tyrosine uptake before the iodoacetate block. Another unexpected observation is that arsenate, although having no effect by itself, counteracts the loss of K⁺ induced by iodoacetate in *Uvra* (Scott and Hayward, 1954); perhaps arsenate stimulates the formation of pyruvate through a partial iodoacetate block and allows more energy to be derived from the cycle.

**Effects of Light on Transport Inhibition in Photosynthetic Organisms**

Iodoacetate causes a marked fall in cell K⁺ and comparable gain in Na⁺ in *Uvra* in the dark (Scott and Hayward, 1953). However, in the light iodoacetate has no effect on K⁺, even at concentrations higher than those caus-
ing marked effects in the dark, and has a minimal effect on Na⁺ (Fig. 1-11). These results were interpreted in terms of a Na⁺ pump operating from glycolytic energy, the photosynthetic formation of phosphoglycerate in the light counteracting the block by iodoacetate. This suggests that the ATP from the 3-phosphoglyceraldehyde dehydrogenase step is not necessary for transport and that energy can come from the metabolism of phosphoglycerate, possibly through pyruvate and the cycle. Similar results have been reported with the red alga Porphyra perforata, except that here iodoacetate causes only a loss of K⁺, cell Na⁺ remaining almost unchanged (Eppley, 1958).

In the light the loss of K⁺ is much less. The failure to gain Na⁺ during inhibition is not a characteristic of this organism, since other inhibitors (cyanide, arsenite, and p-chloromercuribenzoate) cause a significant uptake of Na⁺ as the K⁺ is lost, so that iodoacetate might be thought of as exerting some more specific action.

Transport across the Intestinal Wall

Certain sugars and amino acids are actively transported by the intestine. Iodoacetate can be used to differentiate between substances actively and passively transported (Wilbrandt and Laszt, 1933). Treatment of rat intestine with 1.1 mM iodoacetate reduces the amount of glucose lost from the

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Fig. 1-11. Effects of iodoacetate at 1 mM and 5 mM on the K⁺ (broken lines) and Na⁺ (solid lines) levels in Ulva in the light (L) and dark (D).
(Modified from Scott and Hayward, 1953.)
lumen but has no effect on the movement of xylose. Injected subcutaneously (0.12–0.16 mg/g) it tends to bring the absorption rates of all sugars to a common level, which is that of passive diffusion (in the accompanying tabulation, around 21%). In other words, iodoacetate blocks active transport without altering passive diffusion. Iodoacetate was found to depress phosphorylation of glucose in intestinal extracts, and it was postulate that this is the mechanism of the inhibition in the intact tissue. These results and conclusions have been questioned by later workers. Westenbrink (1936) found that iodoacetate inhibits the absorption of both glucose and xylose from the intact rat intestine, and felt that the site of action is not the metabolism of the intestinal mucosa but the intestinal circulation. Klinghoffer (1938) also found that iodoacetate given subcutaneously (0.06–0.2 mg/g) reduces the absorption of glucose, xylose, and NaCl to approximately equal degrees, and concluded that the action is nonspecific since pylorospasm, hemorrhagic enteritis, and other evidence of intestinal damage were observed. Öhnell and Höber (1939) reported that 0.1–0.16 mg/g iodoacetate given subcutaneously to rats leads to spastic contractions of the intestine, accelerated mucus secretion, and damage to the epithelial villi. However, glucose absorption is inhibited a good deal more than that of xylose. These discrepancies have never been resolved.

Further studies on the characteristics of the iodoacetate inhibition of glucose transport have not simplified the picture. Laszé (1939) found that injections of NaCl previous to iodoacetate completely prevent the inhibition of intestinal glucose absorption. Since the relation between iodoacetate action and NaCl has not been examined in isolated intestine, it is impossible to decide whether this is a local or a systemic phenomenon. Another strange observation, that iodoacetate counteracts the depressant action of 2,4-dinitrophenol on glucose absorption, was made by Rummel et al. (1958). An inhibition of 88% is given by 0.1 mM 2,4-dinitrophenol alone and 50% by 1 mM iodoacetate alone; however, in the presence of both inhibitors the inhibition is 61% — thus iodoacetate stimulates transport when it is inhib-

<table>
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<tr>
<th>Sugar</th>
<th>Relative normal % absorption</th>
<th>% Absorbed</th>
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<tr>
<td></td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>Galactose</td>
<td>115</td>
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<tr>
<td>Glucose</td>
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<tr>
<td>Fructose</td>
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<td>Arabinose</td>
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<td>21.0</td>
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EFFECTS ON PERMEABILITY AND ACTIVE TRANSPORT

EFFECTS ON PERMEABILITY AND ACTIVE TRANSPORT

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glucose resorption in perfused frog kidney, iodoacetate given to frogs, rats, and rabbits at a dose of 0.1 g/kg kills the animals before detectable glucosuria occurs (Walker and Hudson, 1937). The transport of p-aminohippurate is also readily inhibited by iodoacetate (Fig. II-1-18) (Farah and Rennick, 1956); at 0.3 mM the inhibition is 86%, although the respiration is reduced only 30% (Cross and Taggart, 1950), even though 10 mM acetate is present.

The effects of iodoacetate on renal ion transport have not been investigated in much detail, unfortunately. Rabbit kidney slices, incubated in 0.15 M NaCl to lower the K+ content and then placed in 10 mM K+ medium with acetate, reaccumulate K+ and extrude Na+. Iodoacetate at 0.33 mM inhibits this almost completely (see accompanying tabulation) (Mudge, 1951). However, anaerobically iodoacetate at 1 mM has no effect on intracellular K+ or the rate of K+ exchange, indicating that K+ transport is not directly related to glycolysis (Mudge, 1953), so that the aerobic inhibition is presumably due to a block of pyrerve formation. Different regions of the kidney exhibit different patterns of metabolism. The inner zone of the medulla of the dog kidney has a high rate of anaerobic glycolysis and a relatively low respiratory rate, and can reaccumulate K+ anaerobically, whereas the renal cortex cannot (Kean et al., 1961). Iodoacetate at 1.7 mM not only prevents this influx of K+ but causes a further loss of K+ from the inner medulla. However, it is impossible to attribute the inhibition to an action on the EM pathway in either rabbit or dog kidney, since the site of action may be the transport system itself, especially in view of the fact that acetate was present aerobically in the work of Mudge, and the concentration of iodoacetate used by Kean et al. is quite high.

The effects of iodoacetate and iodoacetamide on renal function in intact animals are complex and depend markedly on the concentration of the inhibitor reaching the kidney. Either decreases or increases in the rate of urine formation may thus be observed. When iodoacetamide is infused into the renal artery of the dog, these differences are very clearly shown (see accompanying tabulation) (Strickler and Kessler, 1963). The selective effects on certain transport systems are also evident. The results of Herms and Malvin (1963) are different in some respects, although intraarterial infusion into dogs was also the means of introducing the inhibitor. Iodoacetate was
the kidney were undoubtedly different, although it is difficult to compare these because Herms and Malvin injected the iodoacetate at 0.06-0.124 used instead of iodoacetamide, however, and the concentrations reaching

<table>
<thead>
<tr>
<th>% Change from iodoacetamide at:</th>
<th>0.08 mM</th>
<th>1.6 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine volume</td>
<td>−33</td>
<td>+181</td>
</tr>
<tr>
<td>$C_{Cr}$</td>
<td>0</td>
<td>−9</td>
</tr>
<tr>
<td>$C_{PAH}$</td>
<td>−8</td>
<td>+11</td>
</tr>
<tr>
<td>$UV_{NA}$</td>
<td>−26</td>
<td>+164</td>
</tr>
<tr>
<td>$UV_{K}$</td>
<td>−9</td>
<td>+49</td>
</tr>
</tbody>
</table>

mg/kg/min. They observed no consistent changes in urine volume and little effect on $Na^+$ or $K^+$ transport, except for a marked reduction in $K^+$ secretion in the collecting ducts. Angielski et al. (1960 a) injected iodoacetate into rats at a dose of 0.11 millimole/kg and found the urine flow to be 30–40% reduced for several days, in contrast to the effects produced by the mercurials (see Table 2-6). The marked aminoaciduria, so characteristic of the actions of the mercurials and maleate, is also not seen with iodoacetate.

**Ion Transport through Frog Skin**

A net transport of NaCl and water across frog skin can be demonstrated, and simultaneously there is some active accumulation of $K^+$ intracellularly. The inhibitions of net transport and accumulation do not necessarily run parallel. The effects of iodoacetate are shown in the accompanying tabulation and in Fig. 1-13 (Huf et al., 1957). Iodoacetate belongs to that class of

<table>
<thead>
<tr>
<th>% Change due to iodoacetate at:</th>
<th>0.1 mM</th>
<th>1 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Net transport of NaCl</td>
<td>−21</td>
<td>−48</td>
</tr>
<tr>
<td>Net transport of water</td>
<td>−23</td>
<td>−66</td>
</tr>
<tr>
<td>Cellular $(Na^+)/(K^+)$ ratio</td>
<td>+7</td>
<td>+89</td>
</tr>
<tr>
<td>Electrical potential</td>
<td>−20</td>
<td>−100</td>
</tr>
<tr>
<td>Respiration</td>
<td>−7</td>
<td>+2</td>
</tr>
</tbody>
</table>

inhibitors which depress net transport somewhat more readily than $Na^+$ extrusion and does not significantly inhibit respiration. Contrary to the results in other tissues, it also causes a somewhat greater $Na^+$ uptake than
K⁺ loss, as can be seen in the figure. It is possible to explain at least a major part of these effects on the basis of an increased permeability to the ions and not necessarily an inhibition of active transport, but there is no way to distinguish these mechanisms here.

Fig. 1-13. Effects of various inhibitors on the ions of frog skin. The solid lines represent Na⁺ and the dotted lines represent K⁺. DNP = 2,4-dinitrophenol; IA = iodoacetate; and CN = cyanide. (From Huf et al., 1957.)

Uptake of Cobalt by the Tissues of the Mouse

An interesting study of the effects of iodoacetate on the uptake and distribution of Co⁶⁰ in various organs of the mouse has been made by Maynard (1958). Although the results are not easy to interpret, nevertheless this type of investigation may be quite useful eventually in increasing our understanding of the mechanisms by which substances are distributed in the whole animal. It was first established that 0.1 mM iodoacetate inhibits the uptake of Co⁶⁰ in mouse kidney slices and this was followed by a thorough study of the in vivo response. Iodoacetate was injected intraperitoneally (0.5 millimole/kg) and a few minutes later Co⁶⁰ was given by the same route; the animals were sacrificed at 24 hr. The results are shown in Table
1-33. Maynard concluded that iodoacetate markedly increases the uptake of Co$^{60}$ in most organs and that the effects relate to SH groups in the cell membranes. I would interpret the results quite differently. Since iodoacetate increases the Co$^{60}$ in the blood by 57%, it seems likely that an increased organ uptake would result from this. Therefore I calculated the organ/blood ratios and it can be seen that in only two tissues is there an increase, and these increases are small and may not be significant. In two organs — heart and brain — it appears that iodoacetate inhibits the uptake of Co$^{60}$ around 50%. It may also be mentioned that Maynard found cysteine to decrease the over-all uptake of Co$^{60}$ in all organs, but here the blood level is reduced by over 90%, so that one cannot assume a direct effect on the tissues; it is likely that cysteine reacts with most of the Co$^{60}$ at the site of injection. With respect to iodoacetate, a local inflammatory reaction in the peritoneum may have accounted for the increased absorption of Co$^{60}$.

**EFFECTS ON SKELETAL MUSCLE**

The earliest investigators of the actions of iodoacetate and related compounds emphasized the paralysis and subsequent rigor of voluntary muscle; it was soon demonstrated that the action is directly on the muscle. The development of the initial concepts of iodoacetate rigor has been described in the introduction to this chapter. A muscle stimulated to contract in the presence of iodoacetate slowly weakens and loses its excitability, and this is followed by the development of contracture. The initial changes occur simultaneously with a depression of glycolysis, which eventually leads to a depletion of the high-energy phosphates in the muscle. The glycolytic depression generally has no immediate effect on contractility, and the muscle contracts well as long as it has sufficient ATP. This is reflected in the dependence of the rate of onset of rigor on the degree of activity. Thus, a resting muscle may require hours before rigor occurs, whereas with rapid stimulation a few minutes may suffice. The usual course of iodoacetate action may be represented as in Fig. 1-14. The development of contracture is usually irreversible once it has begun, and indeed contracture often occurs even though the iodoacetate is removed from the medium previously, indicating that contracture is the end result of a series of changes initially induced by the primary action of iodoacetate. Despite the fact that the earliest interest in iodoacetate centered around the modifications of the state of voluntary muscle, the mechanism by which contracture is produced remains unknown.

**Pattern of a Single Contraction**

Attention has been concentrated mainly on iodoacetate rigor and little study has been made of the alterations of the normal contraction before
<table>
<thead>
<tr>
<th>Organ</th>
<th>Per cent dose/gram tissue</th>
<th>Organ/blood ratio(^a)</th>
<th>Per cent dose in whole organ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Iodoacetate</td>
<td>% Change</td>
</tr>
<tr>
<td>Liver</td>
<td>2.52</td>
<td>3.96</td>
<td>+57</td>
</tr>
<tr>
<td>Kidneys</td>
<td>2.30</td>
<td>3.15</td>
<td>+37</td>
</tr>
<tr>
<td>Pancreas</td>
<td>2.11</td>
<td>2.46</td>
<td>+17</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.89</td>
<td>1.56</td>
<td>+75</td>
</tr>
<tr>
<td>Heart</td>
<td>1.91</td>
<td>1.49</td>
<td>-22</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.38</td>
<td>0.59</td>
<td>+55</td>
</tr>
<tr>
<td>Lungs</td>
<td>1.64</td>
<td>2.86</td>
<td>+74</td>
</tr>
<tr>
<td>Brain</td>
<td>0.18</td>
<td>0.14</td>
<td>-22</td>
</tr>
<tr>
<td>Blood</td>
<td>2.47</td>
<td>3.88</td>
<td>+57</td>
</tr>
</tbody>
</table>

\(^a\) The organ/blood ratios were calculated from the values for per cent dose per gram of tissue. (From Maynard, 1958.)
rigor occurs. Lundsgaard (1930 a, d) emphasized that during treatment with iodoacetate, at a time when lactate formation is blocked but before contraction has begun, contractions may be quite normal, indicating the unimportance of lactate for the contraction. Furthermore, the over-all configuration of the contraction — latent period, duration of contraction, and maximal tension — is not changed by glycolytic block.* (Henriques and Lundsgaard, 1931). The phenomenon of treppe is not only evident in poisoned muscles but may be even more pronounced (Lindenbergh, 1932; Lippay and Patzl, 1935). A comparison of normal and iodoacetate-treated frog muscle is shown in Fig. 1-15. Iodoacetate somewhat prolongs the relaxation following electrical stimulation of diaphragm (Kuschinsky and Lüllmann, 1954), and yet reduces or abolishes the prolongation of contraction induced by veratrine (Martensson, 1935). The increased treppe may be only an expression of the developing contracture. The magnitude of the postrest contraction seems to be slightly reduced.

* The contractile tension will eventually decrease, of course, at a rate depending on many factors, but the point here is that normal contractions can occur at a time when the EM pathway is markedly inhibited.
The only thorough investigation of the effects of iodoacetate on muscle contraction is that of Sandow and Karczmar (1950) and is essentially confined to the latent period. A muscle relaxes slightly before an increase in tension begins (Fig. 1-16), and Sandow has described these changes in terms of the three cellular processes indicated in the figure. A brief tetanus applied to a frog muscle leads to a state in which normal stimuli induce a greater than normal tension and the latency relaxation (R) is depressed; it requires 30–45 min for the muscle to return to normal (Fig. 1-17). These alterations may be associated in part with changes in the intracellular pH brought about by the disturbed metabolism and the breakdown of ATP and creatine-P. If a muscle is treated with iodoacetate (0.068 mM), the rise in posttetanic tension is less and the tension soon falls below normal, and progressively decreases to zero as rigor develops. The latency relaxation does not return toward normal but decreases further and eventually disappears. One may say that the effects of tetanic activity on the latent period characteristics are greater in the iodoacetate-treated muscle.

With respect to the time relations of these processes, iodoacetate produces characteristic changes (Fig. 1-17). The latency for tension output (L) is decreased by iodoacetate and does not return to normal during the post-tetany period, but the relaxation latency (Lr) is unchanged by either tetany or iodoacetate for around 7 min, although in the iodoacetate-treated muscle it then increases markedly. If one equates Lr with the LR (latency

![Fig. 1-16. Latent period changes of skeletal muscle, according to Sandow and Karczmar (1950). L = latent period; Lr = mechanically quiescent latency relaxation induction; and R = latency relaxation.](image-url)
relaxation) induction process, which is essentially what is often called excitation-contraction coupling, iodoacetate slows this process; this is not due to a rise in pH, which has the opposite effect, but may be related to the fall in ATP concentration. The difference \( L - L_R \), which is the time for relaxation, is decreased by iodoacetate; this shortening is interpreted in terms of

![Graph showing effects of iodoacetate on muscle contraction](image)

**Fig. 1-17.** Effects of 0.068 mM iodoacetate on the contractile characteristics of muscle using maximal tetani of 3 sec duration. The symbols are the same as in Fig. 1-16. (From Sandow and Karczmar, 1950.)

a more rapidly developing tension induction process (which is rather surprising), due perhaps to the effects of a rise in pH on ATPase activity. There is some question whether the pH changes occurring are of sufficient magnitude to produce any of the effects observed. It should be noted that iodoacetate does not significantly affect latent period behavior unless there is a period of tetanic activity.

**Muscle Rigor Produced by Iodoacetate**

Various substances and types of metabolic disturbance can produce a state of rigor in muscle, but iodoacetate remains the classical and most
reliable inhibitor for this purpose. Some of the characteristics of this rigor in skeletal muscle will be described in this section, and in subsequent sections rigor in other types of muscle will be treated, following which the various theories of iodoacetate rigor will be summarized.

(A) Mechanical characteristics of rigor. The muscle treated with iodoacetate changes from a soft-sticky to a dry-hard state, just as in post-mortem rigor (Bendall, 1960), and there is a loss of extensibility, plasticity, and excitability. Although the shortening of an unloaded muscle in rigor is the same as in maximal isotonic tetanus (around 60%), the tension developed under loaded or isometric conditions is less than in a tetanus (Sandow and Schneyer, 1955). Spontaneous iodoacetate rigor in unstimulated muscle generates 16–17% of maximal tetanus tension, while activity rigor in stimulated muscle generates 35–40% of maximal tetanus tension; the latter is approximately equivalent to the twitch tension. The tension developed in iodoacetate rigor may be a good deal more than in post-mortem rigor (Lippay and Patzl, 1935). Rigor tension is dependent on a number of factors — especially the nutritional, metabolic, and contractile activities of the muscle previous to rigor — but is generally not dependent on the iodoacetate concentration, i.e., when rigor begins to develop it proceeds to completion in an all-or-none manner. However, the kinetics of rigor vary with the iodoacetate concentration. The initiation of rigor follows a lag period which depends on the rate of penetration of the inhibitor into the muscle, the rate of reaction within the muscle cells, the level of high-energy phosphate bonds present, and the rate at which ATP is being used or hydrolyzed. The higher the iodoacetate concentration, the shorter this lag period up to a point. Thus the lag period in frog sartorius is around 10 min at 0.2–1 mM iodoacetate (and presumably this would not be decreased at higher concentrations), 45 min at 0.1 mM, and 180 min at 0.02 mM (Sandow and Schneyer, 1955). Once rigor begins it develops at about the same rate except for the lowest concentration of iodoacetate, where it is definitely slower. The maximal shortening reached is the same in each case.

It has been known since the earliest studies that the lag period for rigor depends on the activity of the muscle, and rigor has been classified into different types on this basis. Lippay and Patzl (1935) stated that there are three kinds of rigor: (1) that of intact muscle with blood supply and innervation, with a lag period around 1 hr, (2) that of muscle stimulated to contract through its nerve, with a lag period of several minutes, and (3) that of denervated unstimulated muscle, with a lag period of several hours. Sandow and Schneyer (1955) similarly classified rigor into spontaneous rigor and activity rigor, depending on whether the muscle is resting or contracting following exposure to iodoacetate. Indeed, a muscle that is fatigued before exposure to iodoacetate will pass into rigor much more rapidly than a normal muscle. Although the time relations may be quite different under these
various conditions, the state of rigor produced is likely to be the same in each. The basis for the kinetics of rigor is metabolic and must be conceived of in terms of changes in a fairly complex multienzyme system (page 227).

Fig. 1-18. Effects of temperature changes on the relaxation of frog muscle in rigor from treatment with iodoacetate. (From Aubert and Rombaut, 1953.)

(B) Reversibility of iodoacetate rigor. Initially it was believed that the rigor produced by iodoacetate cannot be reversed, and this is certainly true to the extent that the muscle cannot be restored to its normal length and excitability. Furthermore, an unloaded muscle will remain in rigor indefinitely (probably until autolysis). However, if the muscle is loaded, it will spontaneously and slowly relax from the state of rigor (Lippay and Patzl, 1935; Aubert and Rombaut, 1953; Sandow and Schneyer, 1955). Frog muscle suspended in 0.4 mM iodoacetate under tension goes into contracture fairly rapidly, but relaxation soon occurs; the rate of relaxation is dependent on the temperature (Fig. 1-18) (Aubert and Rombaut, 1953), possibly indicating some reaction or process upon which relaxation is based, and is more rapid when the muscle is well washed (Lippay and Löning, 1935), which was interpreted to mean that some diffusible substance is formed in
the muscle and responsible for the rigor. Relaxation from rigor has been studied most thoroughly by Sandow and Schneyer (1955) and is one of their evidences that rigor involves a cyclical process like a normal contraction, rather than being entirely different. They believe that rigor is due to the activation of some contractile process instead of the development of the inability to relax. One important question that remains unanswered is: what is the state of the pulled-out extended muscle? It is inexcitable, but can it be contracted by any means? Is it neither contracted nor relaxed, but in a third state, which we might call extension? Is rigor relaxation simply a mechanical luxation of intracellular bonding on a molecular level? It appears that more work must be done before one can accept that this type of relaxation is comparable to that from a normal contraction.

(C) Certain metabolic characteristics of iodoacetate rigor. The problem of high-energy substances, EM pathway intermediates, and the accumulation of various substances will be taken up later, but here it is pertinent to inquire into several more general metabolic relationships. For example, is the development of rigor dependent solely on a block of the EM pathway? Very little study of the effects of pyruvate on rigor has been done. Grimlund (1936) showed that pyruvate and lactate prevent, or appreciably delay, the rigor of muscles in 1 mH bromoacetate, whereas succinate, fumarate, and glycerophosphate have no effect, but I know of no work in which pyruvate or lactate has been added after rigor has occurred. What slight evidence we have thus suggests that at least the onset of rigor is due to a glycolytic depression.

If this is so and the basis for rigor is metabolic, one might expect certain other inhibitors also to induce rigor. Anyone who has used a number of inhibitors on muscle gets the impression that iodoacetate is particularly prone to cause rigor, but this may be due only to the fact that iodoacetate is able to suppress more effectively all the energy-supplying processes. Fluoride should also produce rigor and indeed Lipmann (1930) has shown that in frog muscle it does; it does not so readily in cardiac muscle (see page 221). There seems to be no mention of rigor from 2-deoxyglucose, but the block in most cases is perhaps not complete enough. Mercurials and 2,4-dinitrophenol may bring about rigor of frog muscle (Krueger, 1950; Kutscha, 1961). The rigor of rat diaphragm induced by 2,4-dinitrophenol is very marked; the maximal shortening is greater than in a single contraction, develops quite rapidly, and is reversible (Barnes and Duff, 1954). However, in other muscles, as the heart, 2,4-dinitrophenol is occasionally rather ineffective in causing rigor, and we shall delay final decision on this matter until we have discussed these other tissues.

The metabolic changes occurring during the development of rigor have not yet been generally characterized. Wright (1932) reported some rise in the respiration following the onset of rigor, but claimed that this does not
occur if lactate formation is completely inhibited, and Ronzoni and Ehrenfest (1936) found that muscles treated with 0.24 mM iodoacetate maintain reasonably normal respiration for about 100 min, but that an increase is seen when rigor is initiated. This must mean that rigor sets off some process whereby oxidations are accelerated; it is unlikely that the mechanism is ATP depletion, since there is usually a marked fall in ATP before rigor begins (page 204). The excess respiration is not necessary for rigor, inasmuch as rigor occurs anaerobically, so that the energy for rigor, if energy is needed, does not arise from these oxidations. There is no evidence that much, if any, ATP is formed by these oxidations, so the electron transport systems may be in an uncoupled state. If 2,4-dinitrophenol is added to iodoacetate-treated muscles, there is marked acceleration of the respiration, but rigor occurs even earlier.

(D) Potentiation of rigor. It is well known that the effects of iodoacetate on muscle, including the development of rigor, are accelerated and made more severe by anoxia (Ellis and Beckett, 1954) or cyanide (Hsu, 1950), and the obvious explanation is that muscle anaerobically is fully dependent on glycolysis. If rigor is associated with depletion of creatine-P and ATP, as some claim (page 204), 2,4-dinitrophenol should be a particularly effective potentiating agent, and Cori and Cori (1936) have found this to be true. Acetylcholine and K+ produce a rapidly developing rigor* and both accelerate the onset of iodoacetate rigor (von Ledebur, 1932 b; Fleckenstein et al., 1950). However, this does not indicate direct relationship in the actions of these substances, and the addition of acetylcholine or K+ may merely stimulate the muscle to greater activity, as in a tetanus produced through stimulation of the motor nerve.

(E) Effect of iodoacetate on heat production in muscle. The initial heat of contraction is not altered by iodoacetate or bromoacetate, but the aerobic or recovery heat may be affected (Fischer, 1931; Cattell et al., 1931). The early discrepant results on this point can probably be explained on the basis of different doses or concentrations used. For example, Fischer (1931), using fairly high concentrations of bromoacetate (1.8–7.2 mM), found that the poisoned muscles exhibit the same heat production aerobically as normal muscles anaerobically, indicating complete depression of aerobic heat production. Cattell et al. (1931), however, found that 0.22 mM iodoacetate does not appreciably alter the heat production, but causes it to occur somewhat later after the contraction and to develop more slowly. It depends

* There may be some doubt if "rigor" is the correct term for this state, inasmuch as it probably differs from post-mortem or iodoacetate rigor, and the term "contracture" may be more appropriate. Acetylcholine and K+ depolarize the muscle cell membranes and in this respect the contracture may be much more comparable to a normal twitch contraction.
fundamentally on whether the respiration is depressed markedly or not; in the range in which glycolysis is inhibited and respiration is not, the recovery heat is little changed. Saslow (1936) stated that the recovery heat remains normal as long as the respiration is not reduced to below 50% of normal. Resting heat production is the same in normal and poisoned muscle (Hill and Howarth, 1957). When iodoacetate-treated muscles are stimulated, the heat production rises and remains quite high, even after exhaustion and when the muscle is passing into rigor. If such muscles are placed under anaerobic conditions the heat production falls, but rises again when the muscle is returned to oxygen, indicating the presence of oxidative reactions in the poisoned muscle. "One effect of iodoacetate is to interfere with the mechanism by which the energy released in oxidation can be employed in driving the endothermic reactions necessary for functional recovery" (Cattell et al., 1931).

(F) Relation of high-energy phosphates to rigor. There is a progressive fall in muscle creatine-P and ATP during poisoning with iodoacetate, as initially demonstrated by Lundsgaard (1930 a), and the rate of fall increases with rise in the functional activity of the muscle (Briner et al., 1959; Carlson and Siger, 1960). The general behavior was previously discussed (page 99) and a summary of results given in Table 1-15, but here we are concerned primarily with the relation to rigor development. Rigor has been most commonly explained on the basis of depletion of these high-energy substances, but in different ways. The concept that ATP is used for muscle relaxation (Kalckar, 1941) and that ATP is involved in recovery energization, rather than activity energization, can be applied to rigor; the contractile elements cannot relax if there is depletion of ATP. Others believe that ATP is used in the contractile process (including rigor) and that relaxation can be independent of ATP. Whatever the role of ATP in rigor, it is clear that the kinetics of rigor must be dependent on the relative rates at which ATP is formed and broken down, and thus also on the levels of creatine-P. As far as one can determine, iodoacetate depresses the rate of formation and does not directly affect the rate of utilization, which depends primarily on the state of muscle activity and the various ATPases.

It is interesting to note that the onset of stiffening in post-mortem rigor appears to be correlated with the disappearance of ATP, and this has been explained in various ways, such as the formation of rather rigid complexes of actomyosin, normally kept apart by ATP. The decrease in the extensibility does not begin until the ATP concentration falls to around 2 millimoles/g, and then proceeds rapidly; the critical level depends on the intracellular pH. It has also been suggested that the myofibrillatory ATPase plays no important role in the loss of ATP, either before or during rigor (being well inhibited by the Marsh relaxation factor), and that the sarcoplasmic ATPase perhaps is responsible (Bendall, 1960).
There is no doubt that in iodoacetate rigor the ATP and creatine-P are very low or absent (Norpoth, 1931; Sacks, 1939). At the initial onset of rigor the ATP and creatine-P are reasonably low (see the results of Hermans given on page 100), but the levels fall quite rapidly during the development of rigor and thereafter. Hsu (1950) treated muscles with 0.54 mM iodoacetate and 1.2 mM cyanide for 45 min, stimulating every 5 sec, and analyzed for ATP throughout. He found initial levels to be 206 mg%, at the onset of rigor 139 mg%, at maximal rigor 66 mg%, and after a period of rigor 23 mg%. Hsu did not believe that the fall in ATP and the development of rigor are parallel, however, but there is some doubt as to exactly what relationship one would expect. He also postulated that ATP may be utilized in the development of rigor, a point on which there is still no definite information. Indirect evidence that the store of high-energy phosphate determines the rate of onset of rigor is the common observation that stimulation of the muscle to greater activity accelerates the shortening. Frog muscle, for example, which has been treated with 0.4 mM iodoacetate exhibits four phases according to Maréchal (1958): (1) the tetanic contracture during the 10– to 15-sec period of stimulation, (2) a relatively short latent period during which the muscle is completely relaxed, (3) the development and attaining of rigor, the maximal developed tension being perhaps 60% of that generated by tetanus, and (4) the very slow disappearance of the contracture over several hours. In general one may say that the greater the activity of the muscle, the more rapidly will it pass into rigor; furthermore, the rate of development of rigor is markedly dependent on the temperature, being extremely slow and incomplete at 0°, much faster at 18°, and very rapid at 30°, and this may or may not be related to the rate of disappearance of ATP and creatine-P. Other observations are not easily explained on this basis. If the muscle is stimulated during the development of rigor, there follows a temporary relaxation before shortening continues; also if the muscle during contracture is stretched briefly, it relaxes immediately in an exponential manner and the tension does not return to the initial contracture level for 30 min or longer (Maréchal, 1960).

(G) Creatine kinase. One important source for ATP regeneration in most tissues is creatine-P, if the normal oxidative generation of ATP is blocked, and for this reaction creatine kinase is necessary. If iodoacetate inhibited this enzyme, it would presumably induce rigor sooner and more effectively, assuming that ATP depletion is involved in rigor. As we have pointed out (page 36), creatine kinase is often quite sensitive to iodoacetate in vitro, and Mauriello and Sandow (1959) have suggested it as a possible site for iodoacetate action. However, in vivo the enzyme is inhibited much less strongly, perhaps due to protection by substrates present in the cell (Carlson and Siger, 1959, 1960; Padieu and Mommaerts, 1960). It is interesting to speculate that, if this is true, the enzyme might become progressively
more inhibited as the concentrations of its substrates fall, i.e., that in advanced rigor the enzyme could be inactivated. At the present time, however, there is no evidence that this enzyme is primarily involved in rigor.

(H) Muscle membrane potentials and rigor. Depolarization of muscle cell membranes can under certain circumstances produce a reversible contracture, so that it is necessary to inquire if iodoacetate rigor is in any way dependent on depolarization. Iodoacetate unquestionably lowers the resting membrane potential, but unless this lowering is quite marked it could not be construed as the primary causative factor in rigor. Jahn (1935) found that frog muscles in iodoacetate rigor show some depolarization, and likened this rigor to that produced by depolarizers such as acetylcholine, but it is difficult to determine how much reduction in membrane potential occurred. Liu et al. (1948) treated half of a toad sartorius muscle with iodoacetate and determined the potential between this half and the normal half: suspended in air, there was evidence of depolarization, but in Ringer medium this did not occur, although rigor developed. In K+-free medium, iodoacetate produced rigor while the potential was actually somewhat greater than normal (Fig. 1-19). Small to moderate degrees of depolarization have been

\[
\begin{align*}
\text{Fig. 1-19. Effects of } 0.27 \text{ mM iodoacetate on the toad sartorius muscle membrane potential (measured between normal and poisoned halves of the muscle). Potential changes given in absolute figures; i.e., negative values indicate depolarization. (From Liu et al., 1948.)}
\end{align*}
\]
reported by Fleckenstein et al. (1950), Macfarlane and Meares (1958), and Muscholl (1959). Muscholl found about a 60% depression of the potential at maximal rigor, but rigor began early and was well over half developed before the potential began to fall significantly (Fig. 1-20). Godeaux (1949) could detect no general depolarization during the development of rigor.

The most accurate and reliable results were obtained by Ling and Gerard (1949), using transmembrane recording with microelectrodes. Iodoacetate produces a slow fall of the resting potential over 4 hr (from 80 mv to around 64 mv) and during this period rigor develops to its maximal value. The potential rises somewhat and then rather precipitously falls to zero. Inasmuch as a correlation between muscle creatine-P and degree of initial depolarization (characterizing the A potential) was observed, it was thought that reduction in the creatine-P might be responsible for the early fall in the potential. But in any event, all of the work points quite conclusively to the fact that depolarization is not the primary cause of rigor induced by iodoacetate, although possibly in some instances it may contribute.

Muscle action potentials and after-potentials are generally more sensitive to iodoacetate than the resting potential. Positive after-potentials are depressed (Schaefer and Schömerich, 1938), the magnitude of the action potential is reduced (Gluckman and Koff, 1955), and there is a lengthening of the action potential duration, due to a slowing of the repolarization (Mac-

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**Fig. 1-20.** Effects of 2.7 mM iodoacetate on the resting potential and isotonic shortening (rigor) of rat diaphragm. (From Muscholl, 1959.)
farlane and Meares, 1958; Muscholl, 1959). It is doubtful if any of these changes relates to rigor, but they may well play a role in the modifications of twitch pattern. These effects on membrane electrical behavior are of course due to alterations in ion transport and flux rates (see page 174), but the only ion which may be directly involved in rigor is Ca++. Kutscha (1961) has pointed out that rigor in frog muscle is dependent on the external Ca++ concentration, whatever the agent used to induce the rigor. Iodoacetate at 0.2 mM causes contracture when the Ca++ is 15 mM but does not when Ca++ is absent. Furthermore, the contracture is relaxed somewhat if Ca++ is removed from the medium. Since Ca++ is necessary for normal muscle contraction, the relationship here may not be one particularly pertinent to rigor, but it is very interesting and should be pursued. especially since a dependence on Ca++ might provide evidence that rigor is related to contraction. Incubation of frog muscle with 1 mM iodoacetamide for 1 hr has no direct effect on Ca^45 influx or efflux, but the efflux suddenly increases when rigor begins to develop (Bianchi, 1963). These results are contrary to what one might expect from other work, particularly on the heart.

(I) Direct effects of iodoacetate on contractile systems. Inasmuch as iodoacetate reacts with SH groups, and since actomyosin contains SH groups believed to be important in contraction, it is necessary to inquire whether rigor may to any degree be explained by such a nonglycolytic site of action. Bailey and Perry (1947) have emphasized that both the interaction of myosin with actin and the ATPase activity are dependent on SH groups; they also point out that iodoacetamide reacts very slowly with such SH groups. The sluggish reaction of myosin SH groups with both iodoacetate and iodoacetamide has been noted by Needham (1942), Engelhardt (1946), Polis and Meyerhof (1947), and Bárány and Bárány (1959 a). A decrease in the extractable or soluble fraction of muscle proteins brought about by injections of bromoacetate was observed by Embden and Metz (1930), but this need not result from a direct reaction of bromoacetate with the proteins, since it could also presumably derive from a depletion of ATP and the formation of protein complexes. Jacob (1947) found changes in the myosin fractions of rabbit muscle during rigor, whereas other soluble muscle proteins are unaffected, but again the mechanism is unclear. The viscosity and Ca^45 binding of G-actin and F-actin are not significantly changed by iodoacetamide at 2 mM (Bárány et al., 1962). Results with glycerol-extracted muscle fibers are variable. Hasselbach (1953) claimed there to be no inhibition of the contraction upon adding ATP, whereas relaxation is inhibited; Watanabe and Sleator (1958) found no effect on relaxation but an acceleration of the contraction; and Procita (1960) observed some inhibition of the contraction. In any event, fibers contracted with iodoacetate are not relaxable with the usual agents. It is very difficult to decide whether all of these data taken together imply some direct action on the contractile ele-
ments, but considering the resistance of myosin and actin in vitro and the rather high concentrations used in much of this work, it seems unlikely that there is an important direct action in vivo.

(J) Is rigor associated with the accumulation of some substance? Rigor from iodoacetate develops faster when the muscle is suspended in a moist chamber instead of being immersed in medium, and this has been interpreted to mean that some water-diffusible substance is involved (Lippay and Löning, 1935). The accumulation of methylglyoxal in muscles during iodoacetate poisoning had been shown by Lundsgaard (1930 d) and Barrenscheen et al. (1931), which led Lippay and Löning to suspect that this substance might be rigorogenic, but there is no evidence for this and the theory was soon abandoned. The accumulation of ammonia in muscles in rigor was observed early (Embden and Norpoth, 1931; Norpoth, 1931; Mozolowski et al., 1931), and is now known to result from the sequence of reactions:

\[ \text{ATP} \rightarrow \text{ADP} \rightarrow \text{AMP} \rightarrow \text{IMP} + \text{NH}_3 \]

It is simply an expression of the depletion of ATP and is not directly concerned with rigor. The most likely explanation for the differences observed in muscles suspended in air and in a medium is the accumulation of K\(^+\) outside the muscle cells in the former, as Liu et al. (1948) demonstrated (Fig. 1-19), the K\(^+\) depolarizing the muscle membranes and facilitating the development of rigor.

Effects on Neuromuscular Transmission and Acetylcholine Metabolism

Inasmuch as the neuromuscular junction or end-plate is a particularly sensitive site for the actions of many substances and since metabolic disturbance is known to block junctional transmission before axonal or muscle function is significantly disturbed, what little is known of the effects of iodoacetate will be discussed here so that interpretation of in vivo effects can be put on a broader basis. Hajdu and McDowall (1949) noted that whereas phlorizin blocks the junction selectively, no concentration of iodoacetate that depresses transmission without poisoning the muscle can be found. Ellis and Beckett (1954) obtained somewhat different results. Iodoacetate at 0.18 mM blocks the response of the diaphragm resulting from phrenic nerve stimulation before it depresses the muscle directly as long as oxygen is present, and pyruvate is able to reverse the junctional block. Neuromuscular junction effects could thus be of some significance in the action of iodoacetate in the whole animal.

It is possible that junctional depression by iodoacetate involves a reduction in the synthesis of acetylcholine. Data on the sensitivity of the choline acetylase system are given in Table 1-34, and it is evident that the final step in the synthesis could well be a site for the action of iodoacetate. In
## Table 1-34
**Inhibition of Enzymes Involved in Acetylcholine Metabolism**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Iodoacetate (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholinesterase</td>
<td><em>Electrophorus</em></td>
<td>0.5 5–10</td>
<td>Hargreaves (1955)</td>
</tr>
<tr>
<td></td>
<td>electric organ</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Torpedo</em></td>
<td>2.2 24</td>
<td>Nachmansohn and Lederer (1939)</td>
</tr>
<tr>
<td></td>
<td>electric organ</td>
<td>6.6 47</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 88</td>
<td></td>
</tr>
<tr>
<td>Human erythrocytes</td>
<td></td>
<td>10 4</td>
<td>Markwardt (1953)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 10</td>
<td>Mounter and Whittaker (1953)</td>
</tr>
<tr>
<td>Choline acetylase</td>
<td>Rat brain</td>
<td>1 100</td>
<td>Nachmansohn and Machado (1943)</td>
</tr>
<tr>
<td></td>
<td>Squid head</td>
<td>0.05 0</td>
<td>Reisberg (1954)</td>
</tr>
<tr>
<td></td>
<td>ganglion</td>
<td>0.5 35</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5 63</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.5 78</td>
<td></td>
</tr>
<tr>
<td>Cricket muscle</td>
<td></td>
<td>0.01 32</td>
<td>Severin and Shu-Sen (1963)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1 60</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 100</td>
<td></td>
</tr>
<tr>
<td>Cholinesterase</td>
<td>Cobra venom</td>
<td>3.3 20</td>
<td>Chaudhuri (1950 a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 69</td>
<td></td>
</tr>
<tr>
<td>Cat serum</td>
<td></td>
<td>100 Stim 3</td>
<td>Wels and Repke (1947)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500 12</td>
<td></td>
</tr>
<tr>
<td>Human serum</td>
<td></td>
<td>10 5</td>
<td>Mounter and Whittaker (1953)</td>
</tr>
</tbody>
</table>

This assay, choline and acetyl-CoA were used as substrates. In the total reaction as it occurs in junctions, ATP is required for the formation of acetyl-CoA, so that a second site, the generation of ATP, could also be involved. The results on the cholinesterases in the table show quite conclusively that iodoacetate could not very well disturb junctional transmission by inhibition of these enzymes at the concentrations generally used for blocking the EM pathway or occurring in vivo.
EFFECTS ON THE HEART

It was natural that the interest aroused by the report of Lundsgaard on voluntary muscle, the dissociation of contraction and rigor from lactate formation, would stimulate similar studies on other tissues. Cardiac muscle was one of the first investigated and remains perhaps the most thoroughly studied tissue.* One may say that in general the myocardium resembles voluntary muscle in its response to iodoacetate; i.e., its metabolism is altered similarly, its function is depressed, and eventually a state of rigor occurs. However, there are important differences and much to be learned from the reports on the heart, especially in an analysis of the functional changes. One may ask if there is an obvious difference in the sensitivities of the two tissues to iodoacetate. Ever since the work of Pohl (1888) showing that the heart beats for a short time after a frog is completely paralyzed and in a generalized state of rigor, it has been the impression that the heart is somewhat less sensitive, and most work on isolated preparations tends to confirm this. Nevertheless, administration of iodoacetate to animals invariably results in definite and often marked cardiac changes, as is well shown by the electrocardiographic deviations (page 218); these may be briefly summarized as depressions of contractility and rate, slowing of the conduction, appearance of various dysrhythmias (occasionally terminating in ventricular fibrillation), and systolic arrest.

Effects on the Heart Similar to Those on Skeletal Muscle

Certain changes brought about by iodoacetate in the heart can be conveniently related to the previously discussed results in muscle and repetition of treatment reduced to a minimum.

(A) Potentiation of the effects by anoxia and other inhibitors. The myocardium will continue to function rather well at certain concentrations of iodoacetate if oxygen is present, but will fail rapidly if anoxia is imposed. This is true in all cardiac preparations studied: frog heart (Clark et al., 1932; Gaddie and Stewart, 1933; Kingisepp, 1935), rabbit atria (Chang, 1938 a), dog heart (Bogue et al., 1938), and cat papillary muscle (Lee, 1954). The frog heart can beat for several hours aerobically in 0.27 mM iodoacetate,

* It is interesting, as a sidelight on the pattern of development of inhibitor studies, that work with iodoacetate on the heart has waxed and waned in a very definite way. Two peaks of activity are evident: 1931–1934 and 1953–1955. The rate of such publications by decades reflect: the trends clearly: in the thirties there were 4.5/year, in the forties 0.4/year, in the fifties 2.8/year, and so far in the sixties a fall to 1.0/year. The various factors responsible for this undulating development are many and illustrate one important aspect of the interrelationship of the various fields of science and medicine, but are beyond the purpose of this treatise to describe.
but stops within 7 min if the oxygen is withdrawn (Gaddie and Stewart, 1933). Variation of the $O_2$ tension from 0 to 20 mm Hg produced a steady increase in survival and the rate of beating, but the electrical events are not as sensitive to oxygen lack (Clark and Kingselpp, 1935). The fall of ATP in iodoacetate-treated frog hearts is twice as fast anerobically as aerobically (Clark and Eggleton, 1936), and creatine-P is much more reduced in rabbit atria when anoxia is superimposed on iodoacetate poisoning (Chang, 1938 a). Mammalian hearts are not able to function aerobically when treated with iodoacetate for as long as amphibian hearts, but anoxia still increases the rate of failure. Anoxia depresses the rate of rabbit atria 50% in 21.8 min but in the presence of 0.08 mM iodoacetate this is reduced to 14.2 min; the time for 50% anoxic depression of contractility is likewise reduced from 9.0 to 5.9 min by iodoacetate (Gardner and Farah, 1954). Indeed, anoxia has often been utilized as a criterion for adequate action by iodoacetate; if the preparation fails rapidly during anoxia (i.e., much faster than an unpoisoned preparation), one can be certain that the EM pathway is fairly well blocked and the function aerobically is being maintained by other pathways and substrates. Cyanide also causes rapid failure of hearts treated with iodoacetate (Wertheimer, 1931; Dale, 1937), and fluoroacetate acts similarly (Lee, 1954), as would be expected. These results show that at the proper concentrations of iodoacetate, the heart can derive energy from nonglycolytic sources and that iodoacetate is not markedly inhibiting these oxidative processes.

(B) Effect of functional activity on response to iodoacetate. Most of the preparations studied have been active and failure has been equated with the cessation of this activity. Anoxic failure in the presence of iodoacetate varies linearly with the stimulation frequency, i.e., the rate of energy utilization (Clark, 1935). Quiescent papillary muscles incubated with 0.54 mM iodoacetate for 90 min show normal contractions when stimulation is begun, but they soon fail, the higher the rate of stimulation the more rapid being the failure (Lee, 1954). This relation is seen particularly well in the results of Gardner et al. (1954) on rabbit atria (Fig. 1-12-30). These results are quite like those obtained on skeletal muscle.

(C) Metabolic disturbances produced by iodoacetate. The problems of producing a selective inhibition of the EM pathway in heart have been touched on (page I-877) and it is likely that one can achieve a reasonably specific effect, at least over a certain time interval, if care is taken. It seems difficult, however, to obtain marked changes in cardiac function aerobically with such a specific block, inasmuch as the myocardium usually gets along very well without glycolysis, due presumably to the utilization of lipid, which is indicated by the low R.Q. values (0.74-0.78) generally found in poisoned preparations (Table I-21) (Clark et al., 1937; Burns and Cruickshank, 1937). Thus in our work on the effects of iodoacetate on atrial mem-
brane potentials (Webb and Hollander, 1959), concentrations of 0.2–0.1 mM were found to be ineffective within the time required for accurate determination of changes in the electrical properties and the concentration of 1 mM generally used undoubtedly inhibits the oxidation of pyruvate and fatty acids through the cycle. The respiration of intact preparations is consequently only slightly or moderately reduced by lower concentrations of iodoacetate (0.2–0.5 mM), as shown in the perfused frog heart (Weicker, 1934), the heart-lung (Rühl, 1934; Burns and Cruickshank, 1937), and the cat papillary muscle (Lee, 1954). The respiration of ventricle slices (Webb et al., 1949) and human atrial appendages (Burdette, 1951) is more sensitive, concentrations of 1–2 mM inhibiting well, but here a good part of the effect must be due to depression of the cycle.

The formation of lactate in the heart is reduced, as in skeletal muscle. Early work showed that lactate can generally be utilized by the functioning heart (Evans et al., 1933) and if lactate is exogenously available, as in the heart-lung, its uptake may actually increase (Rühl, 1934; Gottdenker and Rothberger, 1936). The effects of iodoacetate on the uptake and oxidation of lactate and pyruvate by duckling ventricle slices are shown in Fig. 1-9 and have been discussed (page 94) (Miller and Olson, 1954).

The levels of creatine-P and ATP in the heart fall as a result of the action of iodoacetate, but a lack of parallelism between depletion of these phosphates and functional failure has generally been noted (Clark et al., 1932; Clark and Eggleton, 1936). When the frog heart stops beating, whether from iodoacetate alone or with anoxia in addition, the levels are not markedly reduced, but during the development of rigor they fall rather rapidly. Rabbit atria behave similarly according to the data presented on the phosphagen index by Chang (1937), in that at the time of contractile failure the high-energy phosphate has not fallen below 50% of the normal value. The mammalian heart poisoned with iodoacetate utilizes lipid for synthesis of ATP and creatine-P aerobically, and this prolongs the period of activity (Burns and Cruickshank, 1937). There is also a rise in the hexose-P’s, as in skeletal muscle (Weicker, 1934). The metabolic response of the heart to iodoacetate is thus very much like that of skeletal muscle, except for the greater ability of the heart to subsist aerobically by fatty acid oxidation.

(D) Antagonism of iodoacetate depression by substrates. The best method for demonstrating the degree of selectivity of the action of iodoacetate on the heart is to determine the amount of protection or recovery produced by certain substrates. Edwards and Sanger (1933) found that the changes in the refractory period and contractility of turtle atria brought about by 0.32 mM iodoacetate can be partially prevented or reversed by lactate. Parnas et al. (1935) studied the role of P-glycerate in the frog heart and found that it prevents the systolic arrest produced by 0.4 mM iodoacetate, the heart beating strongly and regularly for at least half an hour longer.
than without the P-glycerate. The fact that the heart stops eventually may indicate that the penetration of the P-glycerate may limit its action, or that iodoacetate is acting elsewhere; indeed, that P-glycerate penetrates at all is rather surprising. Pyruvate and lactate increase the contractile amplitude of frog ventricle treated with 1 mM bromoacetate, but Grimlund (1936) found no reversal with succinate, fumarate, or P-glycerate. Clark et al. (1937) tested a variety of substrates on the frog heart poisoned with 0.18 mM iodoacetate, and observed marked recovery with pyruvate, lactate, methylglyoxal, various fatty acids, and certain amino acids, while glucose, succinate, and malate were without effect. These results provide additional evidence that the poisoned heart is able to utilize fatty acids and amino acids. Chang (1938 b) extended these observations to the mammalian heart, finding that rabbit atria depressed by 0.18 mM iodoacetate can be stimulated with pyruvate, lactate, P-glycerate, and propionate, but not by glucose, succinate, fumarate, or malate. In contrast to the frog heart, he found no restoration with amino acids. Masuoka et al. (1952) reported that rat ventricle strips incubated 30 min with 0.2 mM iodoacetate can be restored to near-normal activity by pyruvate but not by glucose, indicating that this concentration is effective in producing a selective block. Identical results were obtained by Covin and Berman (1956).

Other studies have shown that the situation is by no means simple and that the effects of substrates on iodoacetate-poisoned cardiac tissue depend on the concentration of inhibitor, the duration of exposure, the pH, and other factors. Pyruvate has only limited ability to counteract iodoacetate on rabbit atria; marked depression with 1 mM iodoacetate cannot be reversed at all whereas with 0.2 mM iodoacetate partial restoration can be achieved only if the pyruvate is added early before severe depression occurs (Webb, 1950 a). Acetate does not counteract the inhibition by iodoacetate in rabbit atria if it is added later, but may protect slightly if present from the beginning. Yet frog hearts severely poisoned with 5 mM iodoacetate revive gradually when acetate is added, although lactate is more effective (Druckrey and Loch, 1943). Succinate and fumarate do not protect rabbit atria from iodoacetate at pH 7.4, but provide some protection, at least against the reduction in rate, at pH 6.2, indicating that failure to penetrate may explain the ineffectiveness of certain substrates (Wilson et al., 1953).

Iodoacetate depresses the contractile amplitude of rabbit atria much more than the rate, and the antagonism exerted by pyruvate and acetate is more evident on the rate than the contractility (Webb, 1950 a; Wilson et al., 1953). This may well be correlated with the effect of pyruvate on substrate-depleted atria, the rate being restored better than the mechanical function (Webb, 1950 a). Possibly the pacemaker cells possess a different pattern of metabolism from that of the bulk of the atrial cells, as suggested by Wilson et al. (1953). However, other possibilities present themselves:
(1) the pacemaker cells may be more permeable to pyruvate, (2) the energetic basis for impulse discharge may reside in the cycle more exclusively, (3) the pacemaker cells may be less permeable to iodoacetate, which thus acts less on the cycle, allowing pyruvate to be more effective, and (4) the pacemaker cells may possess a lower energy requirement than the rest of the cells (here one must assume that they do not contract) and hence retain a higher level of ATP and creatine-P, so that secondary changes, interfering with the utilization of pyruvate, do not occur. The dependence of the pacemaker cells on the operation of the cycle is also suggested by the fact that fluoroacetate depresses rate and amplitude equally in contrast to the action of iodoacetate (Webb, 1950).

All the cardiac responses to iodoacetate discussed in the preceding section are fundamentally like those of skeletal muscle, except where there is obvious functional difference between the two types of muscle (e.g., the spontaneous discharge in the heart), and there is no doubt that the basic actions of iodoacetate are the same in both.

Effects on Specific Cardiac Functions and Behavior

Iodoacetate reduces both the rate of beating and the contractile amplitude, but usually affects the latter earlier and more strongly (Neuss, 1931; Kleinfeld et al., 1955; Webb, 1950 a). At certain times during the action of iodoacetate, the contractility may be quite depressed and the rate remain normal, or even be greater, as in the perfused frog heart where 0.05 mM iodoacetate slowly increases the rate to some 15% above normal while the cardiac output and stroke volume are depressed 70–80% (Kleinfeld et al., 1955). Gardner et al. (1954) reported that the depression of rate by iodoacetate is always less than the depression of amplitude in rabbit atria at 37–38°, whereas at 30° the opposite is the case, but my work was done at 30° and the rate was much less sensitive. No explanation for this discrepancy can be offered. The frequency-force relationship in rabbit atria is altered by iodoacetate (Fig. 1-21) (Katzung et al., 1957). However, at all rates there is a depression of the basal contraction by iodoacetate, although the degree of inhibition varies somewhat. Iodoacetate also reduces post-stimulation potentiation to the same degree as the basal contraction.

There has been some argument about the relative effects of iodoacetate on the rate of conduction in different regions of the heart, but there is no disagreement that conduction is generally slowed, although it may occur relatively late in the sequence of actions. Electrocardiographic studies have shown a progressive lengthening of the p-r interval (slowing of conduction between atria and ventricles) and a broadening of the qrs complex (slowing of intraventricular conduction) (Siegel and Unna, 1931 a; Goldenberg and Rothberger, 1931; De Boer and Spanhoff, 1933; Lenzi and Caniggia, 1953). This may lead to a complete block between the atria and the ventri-
cles resulting in dissociation, and eventually to serious impairment in conduction over the ventricles. De Boer and Spanhoff (1933) believed that sino-atrial block occurs in the frog heart before atrio-ventricular block, but Goldenberg and Rothberger (1934) felt that the electrocardiogram obtained by the former workers was abnormal, perhaps due to apical damage, and that sino-atrial block is not particularly important. It seems from later work that atrio-ventricular conduction is the most sensitive, but this may depend on the species and many other factors. Anoxia of the frog heart causes slowing of conduction and iodoacetate partially prevents this, which Kingisepp (1935) attributes to the failure of the pH to decrease in the presence of iodoacetate. Studies on isolated atria have confirmed the slowing of conduction. The conduction in rabbit atria is not altered by 0.04 mM iodoacetate for 3 hr but then falls precipitously, following closely the change in refractory period (Fig. 1-22) (Gardner et al., 1954), both of these parameters possibly being related to the depolarization rate. Higher concentrations of iodoacetate and iodoacetamide (0.8–1 mM) produce moderate slowing of conduction in rat atria in a shorter time, but conduction is not as sensitive as the contractility (Webb and Hollander, 1959).

The electrical excitability of rabbit atria decreases fairly rapidly to around

![Fig. 1-21. Effects of various inhibitors on the frequency-force relationship in rabbit atria following 60–70 min exposure. F = 7.2 mM; iodoacetate = 0.05 mM; DNP = 0.03 mM; CN – 0.4 mM; and fluoroacetate = 0.6 mM. (From Katzung et al., 1957.)](image-url)
75% of normal following addition of 0.04 mM iodoacetate, remains at this level for 2 hr, and then falls suddenly along with the action potential (Fig. 1-22) (Gardner et al., 1954). Earlier workers have reported reduction in chronaxie (Gupta, 1933) and even some increase in the excitability as the contractions fail (Van Ginkel, 1934), but in many cases there is confusion between excitability and automaticity. The occurrence of fibrillation penul-

![Fig. 1-22. Effects of 0.04 mM iodoacetate on the various characteristics of rabbit atrium. CR = conduction rate; MSR = maximal rate of stimulation; EE = electrical excitability; AP = action potential magnitude; and DT = developed tension. Since this is only one experiment the results are not very quantitative and since there is no control, the spontaneous changes during this interval are not known. (From Gardner et al., 1954.)](image)

timately (Dobrowolski, 1933; Maltesos, 1934) has prompted some workers to conclude that an increase in the excitability has occurred, which of course is not necessarily so. The refractory period of frog ventricle (Gupta, 1933; Dale, 1935), turtle atrium (Edwards and Sanger, 1933), turtle ventricle (Marshall, 1955), and rat atrium (Lüllmann, 1959 a) is shortened by iodo-
acetate. This is probably related to the shortening of the action potential duration (see page 219), as first suggested by Dale (1935). It is surprising that Gardner et al. (1954) found little if any change in the refractory period of rabbit atrium up to 3-4 hr and then only a sudden increase (i.e., decrease in maximal following rate) (Fig. 1-22); possibly the terminal changes are more related to excitability than refractory period. The dysrhythmias seen both in vivo and in vitro are perhaps to be mainly attributed to the usually marked decrease in the refractory period, coupled with centers of lowered membrane potential and heightened automaticity. Since anoxia causes similar changes one would like to know if there is an effect of iodoacetate on coronary flow, but little work has been done. In the dog heart-lung preparation, iodoacetate causes a marked increase in coronary flow (Gottdenker and Rothberger, 1936), but it is not known if this occurs in vivo.

The alterations of the electrocardiogram observed with iodoacetate are generally those associated with metabolic insufficiency of the myocardium. The lengthening of the p-r and qrs intervals has been mentioned. In addition there is a decrease in the t wave amplitude, inversion, and eventual disappearance in the frog (Lowenbach, 1931; Goldenberg and Rothberger, 1931, 1934; Kossmann, 1957), rabbit (Siegel, 1931; Siegel and Unna, 1931 a; Maltesos, 1934), and dog (Andersen et al., 1955). The behavior of the t wave during metabolic depression by various agents was much studied in the attempt to relate it to myocardial metabolism or to particular pathways. Siegel and Unna (1931 a, b) believed the t wave to be an expression of glycolysis and lactate formation, whereas Lowenbach (1931) felt it to be independent of lactate formation. These arguments are not now of much relevance, since the magnitude and form of the t wave are known to be dependent on the repolarization rate and the pattern of repolarization throughout the ventricle, processes affected by essentially any metabolic disturbance. The t wave inversion seen with iodoacetate is probably not due to coronary constriction but to the direct metabolic deficiency of the myocardium.

**Effects on Myocardial Transmembrane Potentials**

Cardiac function and many of the characteristics discussed in the previous sections depend primarily on the cellular potentials and their changes. Iodoacetate produces alterations generally similar to those occurring during metabolic depression, whether by inhibitors, anoxia, or substrate depletion. Although the electrical changes in atria and ventricles are fundamentally the same, we shall discuss them separately for convenience.

The magnitude of the action potential of rabbit atrium is gradually reduced by 0.04 mM iodoacetate in some cases, but in others there is an initial depression of some 30% followed by a period of no change and then a sudden
fall (Fig. 1-22) (Gardner et al., 1954). A more complete analysis of the membrane potential changes in rat atria was reported by Webb and Hollander (1959) and the results are summarized in Table 1-35. The most sensitive

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Per cent changes from:</th>
<th>Iodoacetate 1 mM</th>
<th>Iodoacetamide 0.8 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15-32 min</td>
<td>5-32 min</td>
</tr>
<tr>
<td>Resting potential</td>
<td>— 0.4</td>
<td>— 6.5</td>
<td>+ 8.5</td>
</tr>
<tr>
<td>Action potential magnitude</td>
<td>— 5.9</td>
<td>— 4.5</td>
<td>— 5.2</td>
</tr>
<tr>
<td>Overshoot (mv)</td>
<td>— 4.23</td>
<td>+ 0.98</td>
<td>— 9.09</td>
</tr>
<tr>
<td>Action potential duration</td>
<td>— 45.9</td>
<td>+ 38.4</td>
<td>— 61.0</td>
</tr>
<tr>
<td>Repolarization rate</td>
<td>+ 70.5</td>
<td>— 32.0</td>
<td>+ 138</td>
</tr>
<tr>
<td>Action potential area</td>
<td>— 47.6</td>
<td>+ 24.6</td>
<td>— 57.0</td>
</tr>
<tr>
<td>Developed tension</td>
<td>— 60.2</td>
<td>+ 14.9</td>
<td>— 58.0</td>
</tr>
<tr>
<td>Developed tension rise time</td>
<td>— 9.9</td>
<td>+ 4.7</td>
<td>— 8.2</td>
</tr>
<tr>
<td>Developed tension duration</td>
<td>— 6.8</td>
<td>+ 8.5</td>
<td>— 10.4</td>
</tr>
<tr>
<td>Conduction rate</td>
<td>— 9.8</td>
<td>— 9.8</td>
<td>— 41.8</td>
</tr>
<tr>
<td>Latent period</td>
<td>+ 6.1</td>
<td>+ 29.3</td>
<td>+ 35.5</td>
</tr>
<tr>
<td>Penetrations (control)</td>
<td>85</td>
<td>55</td>
<td>34</td>
</tr>
<tr>
<td>Penetrations (experimental)</td>
<td>106</td>
<td>57</td>
<td>62</td>
</tr>
</tbody>
</table>

*a The change in the overshoot (the reversal of polarization at the peak of the action potential) is given in millivolts. The original paper should be consulted for the methods of measurement (Webb and Hollander, 1959).

The event is the repolarization of the action potential, and this has also been noted by Lüllmann (1959 a, b). The acceleration of the repolarization shortens the duration of the action potential, reduces the area, and diminishes the degree of potential change imposed on the myocardial cells, which presumably by itself leads to a depression of the contractility, which may fall to quite low levels without significant changes in the resting or action potential magnitudes. The reduction in the overshoot is possibly due to the onset of the faster repolarization. In addition to the shortening of the action potential, there appears to be another factor reducing the contractility, inasmuch as the depression is somewhat greater than predicted on the basis
of the membrane changes, and this may be a result of the intracellular depletion of high-energy phosphate. It is interesting that an initial stimulation of contractility is usually seen (with iodoacetamide it is more pronounced), and this is accompanied by a slowing of repolarization and a lengthening of the duration of the action potential. Changes in the other parameters are in general those expected on the basis of the above effects on the action potential.

The changes observed in amphibian ventricle are quite similar, in that there are relatively minor depressions of the resting and action potential magnitudes, while the repolarization rate is markedly accelerated (Kleinfeld et al., 1955; Kossmann, 1957; Carmeliet and Petit, 1957; Boulpaep, 1959).

The data of Kleinfeld et al. have been plotted in Fig. 1-23 so that the sequence of changes can be visualized. The authors postulated that early effects of iodoacetate may be due to a direct effect on the membrane, perhaps unassociated with metabolism, but quite possibly there are metabolic systems within the membrane. One can conclude that the cellular membrane changes brought about by iodoacetate are very similar in atria and ventricles and that these can to a great extent explain the functional responses
of the myocardium to this inhibitor. It is also clear that these actions are mediated through alterations in the transmembrane ionic fluxes, but we cannot at present say much more than that K⁺ movements are probably modified. Iodoacetate at 0.5 m\(\text{M}\) over a 30 min period causes a significant net loss of atrial K⁺ (126.5 to 121.1 m\(\text{M}\)), K\(^{14}\) efflux being increased 13% and K\(^{14}\) influx being decreased 11%, the contractile depression at 30 min being 86% (Chin, 1963).

Development of Rigor in Cardiac Muscle

Cardiac preparations exposed to iodoacetate go into rigor terminally, either during contraction failure or immediately after cessation of activity, and the picture in general is very similar to that with skeletal muscle. When iodoacetate is injected into an animal the skeletal muscles go into rigor first, the heart continuing to beat for some time; then the ventricles pass into rigor, and the atria may continue beating or be relaxed; and finally atrial rigor occurs (Löwenbach, 1931; Goldenberg and Rothberger, 1931; Wertheimer, 1931). The apparently greater tendency for the ventricles to go into rigor compared to the atria may be due only to the greater amount of work performed by the former and the faster depletion of high-energy compounds. Clark et al. (1932) showed that ATP and creatine-P disappear rapidly during rigor in the frog heart, but Dale (1937) felt that depletion of these substances is not responsible for the rigor, since other inhibitors such as urethane which deplete the cell of energy do not cause rigor. Dale favored the accumulation of some substance. Druckrey and Loch (1943) reported, rather surprisingly, that acetate not only can prevent or delay rigor, but will actually cause some relaxation of a frog heart in rigor. Neither acetate nor pyruvate, nor other substrate, was found to relax rabbit atria in rigor (Webb, 1950 a). Iodoacetate and iodoacetamide are the only inhibitors which regularly produce a marked rigor in rat atria, although arsenite often produces a slowly developing rigor; \(p\)-chloromercuribenzoate never produces rigor, even 2 hr after contractile failure (Webb and Hollander, 1959). Pyruvate has no ability to relax rat atria in rigor, but it is likely, that pyruvate oxidation is interfered with. A complete lack of correlation between rigor and resting potential, or other membrane property, may be noted; some inhibitors markedly reduce the resting potential without producing rigor, while iodoacetate and arsenite lead to rigor which is initiated at a time when the resting potential is definitely depressed (around 25–35%) and contractions have failed, the potential continuing to fall during rigor. Cardiac contractility is augmented by Ca\(^{++}\) and some relationship to rigor might be imagined, but Thomas (1960) found that the presence of EDTA in the medium neither prevents nor reverses the rigor of frog heart induced by iodoacetate. Essentially nothing has been done on the mechanical properties of the myocardium in rigor. We have never observed spon-
taneous relaxation, even though the atria or ventricle strips are under tension.

Recent unpublished work on rat atria by Dr. J. Lacuara will be discussed briefly inasmuch as it sheds light on the mechanism of rigor. Concentrations of iodoacetate from 0.2 mM to 2 mM depress the contractile tension of electrically driven atria progressively over periods of 30 min, complete failure occurring with the higher concentrations at 20 min. Simultaneously one observes the development of rigor and by 30 min it is often quite marked, although there is variation with regard to the rates at which the diastolic tension rises. When 5 mM pyruvate is present with the iodoacetate, the contractile depression is definitely less (not over 30% even with 2 mM iodoacetate) and rigor does not occur. Lactate acts similarly but is somewhat less effective, while acetate is only slightly effective. The time curves for rigor usually consist of two phases, an early phase during which the tension increases to 120–130% of the control value and a later phase during which the tension rises rather suddenly to 170–180% of the control value. The omission of Ca++ in the medium tends to abolish the first phase but has little or no effect on the later rigor. Removal of Ca++ after the development of the initial rigor causes a slow relaxation of the atria, but the later rigor occurs at the same time and to the same degree as in the normal medium. There seem to be two different types of rigor, one dependent on Ca++ and one not. Atrial ATP was determined under various conditions with iodoacetate at 0.5 mM (see accompanying tabulation). Iodoacetate

<table>
<thead>
<tr>
<th>Treatmenta</th>
<th>ATP (μg/g)</th>
<th>Time (min)</th>
<th>% Change of contraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (15)</td>
<td>256</td>
<td>0</td>
<td>—</td>
</tr>
<tr>
<td>Iodoacetate (10)</td>
<td>89</td>
<td>33</td>
<td>— 87</td>
</tr>
<tr>
<td>(to 10% rigor)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iodoacetate (7)</td>
<td>108</td>
<td>36b</td>
<td>— 86</td>
</tr>
<tr>
<td>(10 min after 10% rigor)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyruvate (5)</td>
<td>394</td>
<td>15</td>
<td>+ 17</td>
</tr>
<tr>
<td>Iodoacetate + pyruvate (5)</td>
<td>222</td>
<td>22</td>
<td>— 27</td>
</tr>
</tbody>
</table>

a The numbers in parentheses indicate the number of atria used.

b These atria developed rigor somewhat faster than those in the line above.

causes a severe fall in ATP, which is about 35% of normal soon after the initiation of rigor, but from these preliminary values it seems that the ATP does not continue to decrease as the rigor progresses. In other words, these data suggest that the fall in ATP may be responsible for rigor but that ATP is not utilized in the rigor process. Pyruvate is fairly well able to maintain the ATP level in the presence of iodoacetate and rigor does not occur.
Further ATP analyses at other times must be made before definite conclusions can be drawn as to the role of ATP in atrial rigor.

**Effects of Iodoacetate on the Cardiac Responses to Drugs**

The relationship between the stimulatory action of epinephrine on the heart and cardiac metabolism has been studied quite thoroughly. Neuss (1931) first showed that frog hearts poisoned with 0.72–2.9 mM bromoacetate respond to epinephrine to the same degree as normal hearts, a fact confirmed by Rex-Kiss and Zsadon (1939) for iodoacetate, and for mammalian hearts by Nickerson and Nomaguchi (1950) using 0.5 mM iodoacetamide and 1 mM iodoacetate. However, in the last study, when an adrenolytic drug was present low concentrations (0.01 mM) of iodoacetate antagonized the epinephrine stimulation, whereas iodoacetamide had no effect. This was explained on the basis of two pathways, one blocked by adrenolytics and the other by iodoacetate; epinephrine response is blocked only when both pathways are inhibited. The authors believed, since iodoacetate is active and iodoacetamide is not, that the effect may not be related to reaction with SH groups. On the other hand, Webb (1950 a) found the epinephrine response of rabbit atria to be modified by 1 mM iodoacetate so that the inotropic effect is reduced and the chronotropic effects actually reversed (i.e., epinephrine slows the atria in the presence of iodoacetate). Ellis and Anderson (1951 a) reported that the response of the frog heart to epinephrine is normal as long as iodoacetate inhibits glycolysis only, but when oxidative processes are depressed, so is the epinephrine response; thus stimulation by epinephrine apparently does not involve the EM pathway, and substantiating evidence for this was presented by Ellis (1952). Gardner et al. (1954) also found that iodoacetate does not alter the inotropic response to epinephrine, but shortens and reduces the chronotropic action (no reversal was seen probably because they used low iodoacetate concentrations). Addition of pyruvate, however, restores the chronotropic responsiveness to epinephrine, indicating that pacemaker activity probably depends primarily on energy derived from the cycle. The membrane hyperpolarization produced by epinephrine in dog atria is reduced and eventually abolished by iodoacetate, along with the chronotropic response (Trautwein and Schmidt, 1960). We may thus conclude that epinephrine can stimulate normally the mildly poisoned heart, but that the response is progressively depressed as inhibition develops (perhaps as systems other than the EM pathway are inhibited), until the chronotropic effect is lost or even reversed.

The positive inotropic effect of the cardiac glycosides on the heart is not reduced when the EM pathway is blocked by iodoacetate (Ellis and Anderson, 1951 b; Ellis, 1953 a, b), although Lundin and Ström (1948) noted some lessening of the effect of ouabain in the frog heart by 0.11 mM iodoacetate. The positive chronotropic action of ouabain in embryo chick heart
cultures is not modified by iodoacetate at 0.25 mM (J.A. Smith, 1958). However, the increase in respiration is reduced. The contractile stimulation by Ca\(^{++}\) is also not affected by iodoacetate (Ellis and Anderson, 1951 b).

One might expect metabolic inhibition to reduce to some extent cardiac stimulation by drugs, if only by limiting the reserve of energy for accelerated utilization, but, if the action of a depressant drug is modified, one must assume some more specific mechanism. Iodoacetate was found to alter the response of rabbit atria to acetylcholine in that both the rate and contractility are more readily reduced (see accompanying tabulation) (Webb, 1950 b). This is not the usual effect of metabolic disturbance on the action of acetylcholine (e.g., fluoroacetate significantly diminishes the response of atria to acetylcholine), nor does it seem likely that under these conditions there could be an appreciable inhibition of cholinesterase. Pyruvate augments slightly the negative inotropic effect of acetylcholine, so that one might suggest that fluoroacetate acts here by reducing the oxidation of pyruvate, but for iodoacetate one must assume some mechanism more related to the EM pathway.

### EFFECTS ON SMOOTH MUSCLES

Smooth muscles appear to be less sensitive to iodoacetate than are skeletal and cardiac muscle, but occasionally show a very similar type of response, from which one may perhaps gain additional useful information on the general relationship between contractility and glycolysis. Investigations have been unfortunately sparse except for the intestine.

#### Gastrointestinal Muscle

Neuss (1931) was the first to study the intestine and found it to be fairly resistant to bromoacetate; peristaltic movement is stimulated at 0.72 mM
and higher concentrations, but to produce contracture 720 m$M$ is required. Atropine does not prevent these effects and thus they are not mediated through the vagus. Intestine poisoned with 7.2 m$M$ bromoacetate responds normally to acetylcholine and histamine. Intestinal muscle contains a very poor store of carbohydrate but, when supplied with glucose, exhibits a fairly high rate of glycolysis, which is increased by stimulation of the intestine. One might expect iodoacetate to depress intestine when the metabolic basis for its functioning is glycolytic. Prasad (1935 a) found that 0.54 m$M$ iodoacetate has no effect on rabbit ileum aerobically, but muscle behaves much like skeletal muscle in this respect. It was also shown that glycolysis is well inhibited by iodoacetate at this concentration. Rigor develops soon after spontaneous movement ceases. Pyruvate is able to stimulate iodoacetate-treated intestine, but glucose and lactate are ineffective (Prasad, 1935 b). Prasad also pointed out that the levels of creatine-P and ATP in intestinal muscle are low, so that rigor occurs rapidly when all sources of ATP synthesis are blocked.

Rabbit intestine is stimulated by glucose and pyruvate. Iodoacetate at 1 m$M$ has little effect on the intestine in glucose-free medium, but prevents the stimulation by glucose without interfering with the stimulation by pyruvate (Feldberg, 1943). Indeed, glucose usually causes some depression in the presence of iodoacetate, an effect occasionally observed in other tissues, which could be due to the accelerated depletion of ATP through the formation of hexose-P's.

Although the response of rabbit intestine to acetylcholine aerobically is not prevented by iodoacetate (Porro, 1955), anaerobically it is completely abolished (West et al., 1951). The development of rigor anaerobically can be partially reversed by oxygen, leading to the conclusion that restoration of some ability to form ATP counteracts the process of rigor, although not necessarily reversing it when it is fully developed. Goodman and Hiatt (1964) give the iodoacetamide concentration to prevent acetylcholine stimulation of rat intestine as 5.7 m$M$, at which time 75% of the tissue SH groups are alkylated, this confirming the insensitivity of the intestine; however, interference with the acetylcholine response must occur at much lower concentrations, as shown with other SH reagents.

The tension of guinea pig taenia coli is slowly reduced by 1 m$M$ iodoacetate and eventual failure occurs in a relaxed state (Bülbring and Lüllmann, 1957). There is no dissociation of electrical activity and the tension, the resting membrane potential is not altered significantly, and the spike frequency is slowed and becomes irregular. At low concentrations, around 0.02 m$M$, iodoacetate in common with other inhibitors initially increases the spike frequency (Axelsson and Bülbring, 1961). At this concentration iodoacetate is able to prevent the normal response to a sudden rise in the temperature (disappearance of spikes). Since phosphorylase activity is in-
creased with a rise in temperature, it was postulated that iodoacetate might block this response by its known action on the EM pathway.

Stomach smooth muscle fails quite rapidly in the presence of 1 mM iodoacetate, usually after about 20 contractions when it is spontaneously active, and then passes into rigor (Schmitt, 1935). If the muscle is stimulated electrically, the same sequence occurs but faster. If sufficient iodoacetate is administered to a frog to affect the skeletal muscles, there is little obvious effect on the stomach muscle, but if the muscle is removed it can be readily shown to be definitely altered. It is also known that iodoacetate causes a fall in ATP in the rabbit stomach (Lange, 1955).

Uterus

The guinea pig uterus is stimulated by low concentrations (0.72 mM) of bromoacetate, but passes into rigor at higher concentrations (7.2 mM) (Neuss, 1931). Iodoacetate at 1 mM causes a maximal contraction, which is followed by a paralysis during which the uterus cannot be stimulated by various basic substances normally effective (Klingenberg and Lipp, 1961). The activity of pregnant rat uterus is abolished by 0.2 mM iodoacetate irreversibly and the action potential is reduced, but there is no evidence of depolarization; the response to oxytocin is also lost (Marshall and Miller, 1964). The respiration of rat and rabbit uteri is inhibited 50% by 0.7 mM and 2 mM iodoacetate, respectively (Graubard, 1941). It is evident that we have little knowledge of the effects of iodoacetate on the uterine, but there is no reason for believing it departs markedly from the behavior of other muscles.

Blood Vessels

The results obtained on vascular smooth muscle are conflicting and difficult to interpret. Some workers have found only constriction, for example in the frog or guinea pig during perfusion (Neuss, 1931) or the cat hind limbs (Hitchcock, 1946), rather high concentrations (1–7.2 mM) being used. On the other hand, perfusion of the rabbit ear or guinea pig hind limbs with iodoacetate at concentrations above 1.1 mM produces only dilatation and edema, according to Dobrowolski (1933). Issekutz et al. (1951 a, b) have made the only thorough study of this problem. They perfused the femoral artery of dogs and observed a marked dilatation (blood flow rose from 17–18 to 80–85 ml/min), which was partially reversible, and vasoconstriction eventually occurred. There is some difficulty in deciding whether these changes arise from effects on the vessels directly or are secondary to metabolic alterations in the muscles. Cyanide normally causes vasodilatation, but after iodoacetate it fails to do so and may constrict. A similar effect was noted on the responses to acetylcholine and epinephrine, both of which normally dilate the vessels but after iodoacetate produce more and more
constriction. The dilatation produced by the nitrites is also abolished by iodoacetate but no constriction is seen. These results make it appear that dilatation is an active process requiring energy derived ultimately from glycolysis and oxidations. Although the terminal vasoconstriction may be a form of rigor, there is no evidence on this point.

**THEORIES OF THE MECHANISM OF MUSCLE RIGOR**

The development of rigor following treatment with iodoacetate appears to be a general phenomenon in all types of muscle. Many theories have been suggested but few have survived. On the basis of the evidence presented in the previous sections we can eliminate the following theories from serious consideration: (1) that rigor is due to the accumulation of lactate, (2) that it is due to either a decrease or increase in the intracellular pH, (3) that it is due to the accumulation of methylglyoxal, (4) that it is due to the accumulation of ammonia, (5) that it is brought about by a direct action on the contractile elements, (6) that it is dependent on an increased Ca++ influx, and (7) that it is caused by a depolarization of the muscle membranes. Possibly one or more of these mechanisms contribute in certain instances, but none can explain rigor nor can they be of major importance.

Until the state of the muscle in rigor is much better known, it will be impossible to formulate mechanisms with any degree of certainty. There is still no general agreement on whether ATP functions in contraction or relaxation energization, or on whether muscle in rigor cannot relax or is in a state of constant contractile activation, or whether rigor is related or not to normal contraction. Sandow may well be correct in his comparison of rigor and contraction and in his concept that ATP drives the development of rigor, but the evidence is not satisfactory. One problem often overlooked is that total over-all ATP is determined in the muscle, whereas depletion of compartmentalized ATP may be very important. It is true that rigor may begin when the ATP has fallen only 50%, but that ATP measured may be irrelevant to rigor. There is no evidence at all that rigor obtains energy from ATP hydrolysis; ATP decreases before rigor, during rigor, and after rigor, and for causes unassociated with rigor.

Although admittedly not completely satisfactory, the following mechanism is believed to be the most likely from the evidence at hand, and in general is the one which has been accepted by most workers in the field. The primary action of iodoacetate is on the EM pathway; if an action is exerted on the cycle or oxidations, or if hypoxic conditions exist in the muscle, or if the muscle has a low supply of noncarbohydrate substrate, these will further reduce the energy-supplying reactions. Thus the fundamental effect is an inhibition of ATP generation. Due to the various ATPases and functional activity, ATP is slowly or rapidly hydrolyzed; as this occurs, it is
regenerated from creatine-P. When the supply of creatine-P is exhausted, the ATP falls rather suddenly (but at a rate dependent on the functional state of the muscle); this probably accounts for the many observations that various properties are maintained relatively well over a period and then disappear precipitously. When the ATP normally bound to the contractile proteins, which is assumed to keep them separated and in a mobile state, is sufficiently depleted, the actomyosin system begins to contract, and this continues as the ATP is lost, until a complex multimolecular association has taken place. Relaxation by the addition of ATP may be impossible at this stage, since the ATP may not be able to gain access to its binding sites. The muscle, however, can gradually be lengthened by exerting a force upon it, and the complexes can be slowly broken down; such an artificially lengthened muscle is no longer in a relaxed state or in a contracted one, but in a third state. Such a theory implies no particular role of ATP in normal contractions; it may function either in contractile activation or in relaxation, this being the unbound fraction.

**EFFECTS ON NERVE FUNCTION**

The demonstration that muscle contraction is independent of lactate formation prompted an investigation of the relationship of lactate to nerve function. A brief summary of the metabolic responses of nerve tissue to iodoacetate will be presented before the effects on function are discussed.

Injection of iodoacetate into pigeons in lethal dosage reduces the brain lactate about 50% (Kinnersley and Peters, 1930); injection into dogs produces rather erratic behavior of brain lactate, but in general it is reduced following 10 min incubation after removal from the animal (Haldi, 1932); and perfusion of frogs with 0.7–1.8 mM iodoacetate for 1 hr reduces brain lactate markedly if supplemented with anoxia, anoxia alone somewhat increasing the lactate level (see accompanying tabulation) (Holmes, 1933).

<table>
<thead>
<tr>
<th>Brain lactate (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before perfusion</td>
</tr>
<tr>
<td>Oxygenated perfusion</td>
</tr>
<tr>
<td>Anoxic perfusion</td>
</tr>
<tr>
<td>Anoxic perfusion + iodoacetate</td>
</tr>
</tbody>
</table>

Thus iodoacetate inhibits glycolysis in nerve *in vivo*. Inhibition of anaerobic glycolysis in nerve and brain has been reported several times (Gerard, 1931; Heald, 1953), but respiration seems to be less sensitive when the tissue is intact, the nerve sheath apparently presenting a barrier to the penetration of iodoacetate since, if the sheath is split, inhibition occurs more rapidly
(Chang and Gerard, 1933). Like muscle contraction, nerve function fails quite rapidly when anoxic conditions are superimposed on the iodoacetate inhibition, whereas either alone requires long periods to depress, indicating again that glycolysis is probably the principal source of energy anaerobically (Ronzoni, 1931). The creatine-P is reduced by iodoacetate (Gerard and Tupikow, 1931; Chang and Gerard, 1933; Holmes, 1933) and more rapidly if the tissue is deprived of oxygen. It is interesting that Dahl et al. (1962) reported iodoacetate at 20 mM to reduce the electrical activity in chicken vagus but to have no effect on creatine-P or ATP levels, even though the nerve is stimulated; the periods of observation here were quite brief. Thiocyanate has been reported to fall in stimulated frog nerve poisoned with iodoacetate (Wyss and Wyss, 1945). Lactate can be utilized by iodoacetate-treated nerve, as shown by respiration studies (Chang and Gerard, 1933) and by its ability to prolong nerve function (Fong, 1932). Glucose utilization, however, is markedly decreased as expected, as shown in the perfused frog spinal cord, in which case the R.Q. is reduced from 0.89 to 0.71, respiration being simultaneously depressed (von Ledebur, 1932 a). The respiration of minced rat cortex is depressed by iodoacetate, newborn rats showing a greater sensitivity (46% inhibition) compared to adult rats (26% inhibition) (Tyler, 1942). The endogenous formation of ammonia by brain slices is depressed 42% by 1 mM iodoacetate (Weil-Malherbe and Green, 1955 a), in contrast to muscle where iodoacetate increases ammonia release, but the origin of the ammonia is different in the two situations. Finally, the interesting results of Heald (1953) on electrically stimulated brain slices must be considered. The respiration and glycolysis in stimulated tissue are about double that of resting tissue. The resting respiration and glycolysis are rather insensitive to iodoacetate, whereas the activity metabolism is very sensitive, being almost abolished by concentrations of 0.01–0.03 mM (see Fig. 1-10). If in vitro studies with inhibitors are to be applied to in vivo results, these data show that active tissue should be used. In summary, one can conclude that nerve behaves much like muscle in the presence of iodoacetate metabolically, and one would predict nerve function to be independent of lactate formation, but eventually dependent on the energy derived from the oxidation of pyruvate and the stores of ATP and creatine-P.

Inhibition of conduction in nerve axons presents special problems. First, ion pumps have established high ionic gradients for Na+ and K+ across the membrane and, even though these active transports are blocked, the nerve will conduct for some time due to the fact that only a very small fraction of the cell K+ is lost or Na+ from the medium is gained per impulse. Thus the nerve can operate for extended periods even though no energy is immediately available. Second, the myelin sheath around most nerves which have been studied presents a barrier to penetration of ionic inhibitors, so
that fairly high concentrations must be used to produce significant effects, and in these cases it is difficult to know the intracellular concentration of inhibitor and the specificity of the action.

All of the early studies demonstrated certain fundamental features in the responses of nerve axons to iodoacetate (Ronzoni, 1931; Feng, 1932; Chang and Gerard, 1933; Shanes and Brown, 1942). These may be summarized as follows: (1) there is a lag period of one to several hours before effects are evident, (2) the rate of conduction decreases and eventually conduction fails, (3) there is a slow fall in the resting and action potential magnitudes, (4) lactate and pyruvate are partially effective in counteracting iodoacetate, (5) conduction failure is accelerated by increased activity, e.g., tetanization, and (6) failure is accelerated by reducing the oxygen tension. These results all conform to what would be expected. The report of Lorente de Nó (1947) that iodoacetate and iodoacetamide delay the anoxic depolarization of nerve, in direct contrast to the results of Shanes and Brown (1942) and contrary to the behavior of other tissues, is very surprising, but Liu (1950) repeated this work and found that the odd response was due to a technical error and a lack of controls. Liu under the circumstances could not demonstrate much effect in either direction on anoxic depolarization with 2 mM iodoacetamide but, upon readmission of oxygen, the nerve repolarized more slowly. More recent work has not contributed much of fundamental interest and can be summarized very briefly. Iodoacetate at 5 mM depresses the spike amplitude progressively, and simultaneously there is a decrease in threshold; however, when the spike is reduced 80–90%, the threshold increases markedly (Jenrick, 1957). The resting potential is depressed 30–40% at the time of conduction block. Conduction in the chicken vagus is slowed by 20 mM iodoacetate and the spike height is lessened (Dahl et al., 1964). Anoxia superimposed on iodoacetate produces much more depression, but it is remarkable that no fall in ATP was observed. The posttetanic hyperpolarization in nonmedullated cervical sympathetic nerves is abolished by 1 mM iodoacetate, and usually the after-potential is reversed; other inhibitors do not do this (Ritchie and Straub, 1957; Holmes, 1962). The intraaxonal injection of high concentrations of iodoacetate does not affect the action potential (Brady et al., 1958). Iodoacetamide at 5.4–27 mM has been shown to alter the structure of the myelin sheath, as shown by X-ray diffraction, and this is believed to be due to reaction with SH groups there, but whether this is of significance in the actions on nerve is not known (Millington and Fimean, 1958). The extrusion of Na+ and uptake of K+ are depressed by iodoacetate, which undoubtedly is the basis for the fall in the resting potential (Shanes, 1952). It is interesting that Ca++ can stabilize nerve membranes against a variety of factors, and can counteract the fall in the potential produced by iodoacetate (Shanes, 1942). It would be important to know by what mechanism this is accomplished.
Turning to the effects of iodoacetate on synaptic function, we find a much greater sensitivity compared to axons, due presumably to the greater penetration of the inhibitor. Larrabee and Bronk (1952) recorded pre- and postganglionic action potentials from the stellate and superior cervical ganglia of cats, and found that at a time when the preganglionic spike is reduced 20% by 0.1 mM iodoacetate acting for 40 min, the post-ganglionic spike is depressed 48%. The selective action here is not very great. The spontaneous activity of crayfish ganglia is not affected by 1 mM iodoacetate, indicating that it does not depend on glycolysis (Prosser and Buehl, 1939). Von Ledebur (1932 a) perfused the isolated frog spinal cord with 0.54 mM iodoacetate and within 7 min all reflex activity had been abolished, indicating a potent depressant action on synaptic transmission. After the loss of reflexes, the muscles can still be stimulated through their motor nerves. Holmes (1933) also perfused frogs with iodoacetate and found that 0.7 mM reduces the number of animals convulsing with strychnine. He further showed that animals can still convulse occasionally even though lactate formation is blocked, showing that the activity is not related to glycolysis. The changes in lactate, creatine-P, and function are shown in Fig. 1-24. The superior cervical ganglion of the cat is also quite sensitive, reversible transmission depression occurring at 0.01–0.05 mM iodoacetate,

**Fig. 1-24.** Effects of 0.54 mM iodoacetate on the levels of lactate and labile phosphate in frog brain, and the per cent convulsing after the administration of strychnine. (From Holmes, 1933.)
higher concentrations antagonizing the actions of acetylcholine and K+ (Halász et al., 1960). It was thought that iodoacetate does not interfere with the synthesis or release of acetylcholine but mainly blocks the response of the postganglionic cells.

The hyperpnea induced by iodoacetate in the whole animal led several investigators to study the effect of iodoacetate on the carotid area and associated reflexes. Winder (1937) found that perfusion of the carotid segment in dogs with 0.014–0.7 mM iodoacetate practically abolishes the anoxic response, but that the pressoreceptor reflexes are unaffected. The anoxic reflex may be related to the formation of acid within the receptor cells and possibly iodoacetate inhibits this (Anichkov, 1953). A very interesting study of this problem was made by Landgren et al. (1954), who recorded action potentials from the sinus nerve in cats upon intracarotid injection of varying amounts of iodoacetate. Injection of 2 mg iodoacetate leads to an increase in the number of potentials from chemoreceptor cells but, if the dose is raised to 10 mg, these potentials are quickly abolished while the pressor spikes remain unaltered; lobeline, a potent stimulator of the chemoreceptor cells, is now without effect. They believed that carotid body function depends on an acetylcholine cycle and that iodoacetate inhibits the formation of acetylcholine. The respiratory effects of iodoacetate in the animal probably involve the carotid body, but the exact mechanism of the effect on carotid function is not understood.

EFFECTS ON THE RETINA AND THE EYE

Retinal tissue is of particular interest with respect to its response to iodoacetate because of its very high rate of glycolysis (Table 1-36). Glycolytic activity is much higher in retina than in any other type of tissue, even tumors, embryo, and bone marrow, all noted for their relatively high glycolytic rates. Although the absolute Pasteur effect is also high, the percent Pasteur effect is low (0.49), being similar to tumors (0.52), whereas in normal tissues and embryo it is around 0.83. The high glycolytic activity does not necessarily imply that retinal function will be especially sensitive to iodoacetate, inasmuch as it is the degree of dependence of the function on glycolysis which is important, but it is reasonable to anticipate that, in a tissue with rather unique metabolic patterns and functions, these will be in some manner related.

Retina

Selective impairment of retinal function and damage to the retina have indeed been demonstrated by Noell (1951), but before these problems are discussed it will be well to review in greater detail the metabolism of the
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Respiration $-Q_O$</th>
<th>Aerobic glycolysis $Q_O^{CO_2}$</th>
<th>Anaerobic glycolysis $Q_{N2}^{CO_2}$</th>
<th>Absolute Pasteur effect $Q_{N2}^{CO_2} - Q_O^{CO_2}$</th>
<th>Meyerhof oxidation quotient $[Q_{N2}^{CO_2} - Q_O^{CO_2}] / Q_O$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal tissues (mean)</td>
<td>9.0</td>
<td>2.1</td>
<td>12.0</td>
<td>9.9</td>
<td>3.3</td>
</tr>
<tr>
<td>Spontaneous tumors (mean of 27)</td>
<td>10.8</td>
<td>14.5</td>
<td>30.1</td>
<td>15.6</td>
<td>4.3</td>
</tr>
<tr>
<td>Embryo</td>
<td>12.5</td>
<td>3.0</td>
<td>18.0</td>
<td>15.0</td>
<td>3.5</td>
</tr>
<tr>
<td>Brain (gray matter)</td>
<td>11.0</td>
<td>3.0</td>
<td>19.0</td>
<td>17.0</td>
<td>4.5</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>11.0</td>
<td>3.0</td>
<td>21.0</td>
<td>18.0</td>
<td>4.9</td>
</tr>
<tr>
<td>Retina</td>
<td>31.0</td>
<td>45.0</td>
<td>88.0</td>
<td>43.0</td>
<td>4.2</td>
</tr>
</tbody>
</table>

\[ \text{All tissues are from the rat except for the tumors. The values and means are only approximate and depend on the experimental conditions, but indicate fundamental metabolic characteristics. (Data taken from Table 4 of "The Glycolysis and Respiration of Tumors" by Aisenberg, 1961.)} \]
retina and its modifications by iodoacetate. A connection between glycolysis and retinal function is indicated by the fact that the rate of anaerobic glycolysis in rat retinas suddenly rises around 10 days after birth, at a time when the eyes open (Graymore, 1959). This rise is not the result of an increased energy demand since it occurs when the rats are in total darkness. It has been suggested that the visual cells have a higher glycolytic rate than the rest of the retina (Graymore and Tansley, 1959 b), which means that their activity is even higher than indicated in the table, and it has been shown that doubling of glycolytic rate occurs at the time of visual cell differentiation (Kerly, 1959). The high glycolytic rate implies a relatively high concentration of the enzymes of the EM pathway in the retina, but these have not been studied except for 3-PGDH, which is very high in the outer plexiform layer of the monkey retina, although its concentration falls off in the next two layers and is quite low in the outer rod and cone segment (Schimke, 1957). Thus there is a marked variation in the concentration of 3-PGDH throughout the retina, which may be correlated with different glycolytic activities.

Retinal glycolysis is quite sensitive to iodoacetate. Anaerobic formation of lactate from glucose in cell-free extracts of ox retinas is inhibited 91% by 0.05 m\(M\) iodoacetate (Kerly and Bourne, 1940), in homogenates 97% by 0.23 m\(M\) iodoacetate (Futterman and Kinoshita, 1959), and in intact tissue 50% by 0.05 m\(M\) iodoacetate (Crane and Ball, 1951) (see Table 1-11). Retinal respiration is depressed but is not nearly as sensitive as glycolysis. Inhibition of retinal glycolysis in vivo has also been demonstrated. The intravenous injection of 48 mg/kg iodoacetate into rats leads to a 31% depression of glucose utilization by the retina, without affecting the utilization in either brain or diaphragm (Kornbleuth and Ben-Schlomo, 1956). Indeed, iodoacetate acts surprisingly rapidly, in that injections of 60 mg/kg iodoacetate inhibit anaerobic glycolysis in rat retinas in 10 min by 75%, and this is maintained relatively constant for at least 24 hr (Graymore and Tansley, 1959 b). This dose eventually produces severe retinal damage and degeneration. These results make it unlikely that the pentose-P pathway is very important in retina, and this has been confirmed using glucose-1-C\(^{14}\) and glucose-6-C\(^{14}\) (Futterman and Kinoshita, 1959; Rahman and Kerly, 1961). At the most, one molecule out of four is oxidized through the pentose-P pathway. Nevertheless, there is a high concentration of glucose-6-P dehydrogenase in the retina. It is interesting that \(CO_2\) fixation in retina is apparently stimulated by iodoacetate (Crane and Ball, 1951). It is believed that glucose-6-P dehydrogenase is involved, NADPH being formed for fixation of \(CO_2\) into pyruvate to form malate; when the EM pathway is blocked, more glucose-6-P is oxidized and more NADPH is available. This is a good example of how an inhibitor can stimulate a reaction linked in some manner to the system that is inhibited, although it is doubtful if this action is
of much significance in the effects of iodoacetate on the retina. The uptake and accumulation of $K^+$ in retina depends, as in brain, on the presence of both glucose and glutamate. Iodoacetate at 1 mM abolishes the uptake of glutamate and the formation of glutamine in retina, and simultaneously not only prevents accumulation of $K^+$ but induces a marked loss from the cells (Terner et al., 1950). Such an action may help to explain the reduction of the retinal potentials by iodoacetate.

The close relationship between glycolysis and retinal function is suggested by the excellent work of Noell (1951) at Randolph Field. A rabbit injected intravenously with 15 mg/kg iodoacetate shows a decline in the electroretinogram (ERG) $b$ wave within 20 sec and a decline of the response in the optic nerve; the $b$ wave vanishes in 2 min. The $a$ wave is not affected at this time, but soon declines and after 20 min has disappeared, so that now no response to illumination can be obtained. The minimal effective dose is 2 mg/kg. The dosage used above might be expected to produce a plasma concentration of around 0.5 mM, which could certainly inhibit retinal glycolysis.* Indeed, it has been shown histochemically that retinal 3-PGDH is inhibited quite significantly at the time of extinction of the ERG (For-gacs, 1963). Retinal potentials in the cat are impaired even more rapidly, both ERG waves declining simultaneously and failing within 2 min, but in the frog the rate of action and the sensitivity are less than in rabbits. The effect of iodoacetate on the ERG potentials is different from that of anoxia or general metabolic depression. Recordings from microelectrodes placed in the retinal cells show that the effect of iodoacetate is to impair the excitatory processes activated by illumination. At these dosage levels and times, there are only minor effects on the cardiovascular and central nervous systems. No other vertebrate tissue function seems so dependent on glycolysis, and perhaps the first effect of iodoacetate is to blind the animal. This initial work has been extended in various directions and we shall confine ourselves for the present to the acute effects, leaving the damage and degeneration of the retina for later discussion.

The observations of Noell have been confirmed in several reports and with different species; although species may differ in sensitivity, they all respond in the same basic manner. Along with the $b$ wave depression in

* Since this concentration would be expected to inhibit glycolysis in other tissues as well, the problem comes up of the penetration of iodoacetate into the various tissues Other neural tissues, especially in the central nervous system, are probably relatively impermeable to iodoacetate. The remarkable rapidity with which iodoacetate affects the retina indicates that the retina must be not only very susceptible but also very accessible. The iodoacetate need not enter the cells to produce these membrane electrical changes, but one must assume even here that the rate of reaction with SH groups is much faster than is usually observed. These problems of differential penetrability and selective effects on the retina have in general been ignored.
cats, Bornschein (1958) noted a temporary increase in the spontaneous activity, and suggested that iodoacetate may affect primarily the photoreceptors and that these participate in the spontaneous discharge. Quite possibly the fall in the resting membrane potential of the visual cells (Müller-Limmoth and Blümer, 1957) is responsible for this spontaneous activity, since the lowering of the resting potential within a certain range usually increases excitability and automaticity. The ERG changes resulting from a single intravenous injection of iodoacetate are slowly reversible, the b wave recovering to more than half-normal within 5–6 hr and to normal within 1 day (Noell, 1952). It would be important to know what is involved in this reversibility. The pupillary reflex to light is abolished early, as might be expected, so that Schubert and Bornschein (1951) wondered whether the primary attack is on glycolysis or on the photochemical reactions. Wald and Brown (1951, 1952) found that even high concentrations of iodoacetate (30 mM) do not inhibit the regeneration of rhodopsin, although the mercurials block this very readily. There is thus no evidence that photochemical reactions are directly affected, but the fundamental reactions linking photon absorption with the membrane changes involved in the initiation of impulses have never been studied. Retinal edema is marked after iodoacetate, being maximal in 30 min, and this may result from interference with the active transport of water (Graymore, 1958). It is not known if the swelling is in any way responsible for obliteration of the retinal blood vessels, a mechanism which has been postulated for later degenerative changes, but it seems highly unlikely that it would play a role in the alterations of the ERG. If glycolytic inhibition is responsible for the ERG changes, other inhibitors of the EM pathway might be expected to produce similar effects. Karli (1952) observed no effects of fluoride in rabbits, but it was given by stomach tube and probably did not reach the retina in sufficiently high concentration, whereas Babel and Ziv (1956) found temporary ERG changes from injections of fluoride, although no damage was produced, probably due to the rapid reversal of the inhibition. Other SH agents, such as the mercurials, arsenicals, and o-iodosobenzoate, do not produce these retinal changes, although bromoacetate acts like iodoacetate (Sorsby et al., 1957). These results provide indirect evidence that glycolytic inhibition is the mechanism by which iodoacetate acts. It is perhaps pertinent to note that the endocochlear application of iodoacetate decreases the microphonic potentials in the cat (Bornschein and Thalmann, 1963).

Single intravenous injections of iodoacetate (20–35 mg/kg), or two to three injections (20 mg/kg) over a period of 24 hr, impair the ERG permanently in rabbits and may selectively destroy the rod and cone cells. Karli (1952) observed that several days after iodoacetate injection the rods are severely affected, their nuclei pycnotic, and the chromatin condensed. Following these visual cell changes, he found secondary modifications of
the retina: the retinal neuroglia reacts and numerous mitoses are seen in the internal granular layer, the pigmentary epithelium presents areas of localized proliferation, and some degeneration is evident in the external granular layer. Karli concluded that a pigmentary retinosis is produced secondary to the primary effect on the visual cells. There is a great similarity between the picture produced by iodoacetate and the hereditary human degenerative disease of retinitis pigmentosa, and it is interesting to speculate that some metabolic lesion due to a genic aberration is present in this disease. Pigmentary clumping has been observed histologically (Noell, 1952) and ophthalmoscopically (Lucas et al., 1957), and pigment cell migration (Rabinovitch et al., 1954) and proliferation (Karli, 1954; Babel and Ziv, 1956) also occur.

Noell (1952) described many of the later degenerative changes in greater detail in rabbits, cats, and monkeys, and the following additional observations may be noted. (1) No ERG is detectable after 3 weeks and yet stimulation of the optic nerve gives a normal striate reaction, showing the effect to be all distal. (2) No histological changes can be detected in the optic nerve. (3) The destruction of the visual cells, with nuclear pycnosis and death of the rod cells, occurs less than 24 hr after the injections, showing a fairly rapid killing action on these cells. (4) The cone cells exhibit remarkable changes: a swelling of the ellipsoidal body followed by longitudinal shrinkage, with destruction of all special organelles, so that the cells eventually appear as a row of simple epithelium, as if dedifferentiation had occurred. (5) The cone cells are damaged more and more as their distance from the fovea increases. Some of these effects might be interpreted as due to disturbances in the blood flow, but reduction of the flow to the retina does not induce similar changes. The chick retina, which is quite sensitive to iodoacetate, shows less differential effect, the whole retinal structure being grossly disturbed (Rabinovitch et al., 1954). Selected illustrations of retinal alterations are given in Fig. 1-25.

Electron microscopy studies by Lasansky and De Robertis (1959) demonstrate that lesions of the outer segments of the rod cells occur as early as 3 hr after a single injection of iodoacetate (20 mg/kg), and after 6 hr there are disorganization, vesiculation, and lysis of the rod sacs. The typical multilamellar structure of the rods is disorganized very early and the endoplasmic reticulum shows vacuolization. A second injection leads to complete destruction of the rods, but also destroys cone cells, which are more resistant and do not suffer markedly from a single injection. The authors suggest that iodoacetate affects some system responsible for the maintenance of membranes or lamellae within the cells. Cibis et al. (1957) found the histological lesions produced by iodoacetate to be very similar to those resulting from X-radiation, a comparison also made by Kent (1959), but it is doubtful if it is profitable to extend further these superficial similarities.
I. Iodoacetate and Iodoacetamide

![Illustrations of the effects of iodoacetate on retinal histology.](Image)

**Fig. 1-25.** Illustrations of the effects of iodoacetate on retinal histology.

1. Cone cells in *Macaca mulatta* 5 weeks after the last injection of a 2-week period (20–40 mg/kg iodoacetate). A: Cone cells normal in central region of fovea; B: cones abnormal and rods disappeared in parafovea about 1-mm distance from central area; C: cone cells more extensively damaged 2 mm from central area in parafovea; D: inner segment of cone cells almost completely vanished about 4 mm from central area. (From Noell, 1952.)

II. Retinas of chicks; iodoacetate at 40 mg/kg intravenously. 1: Normal retina; 2: 2 hr after iodoacetate; pycnosis in the ganglionic and inner and outer granular layers; 3: 24 hr after iodoacetate, some glycogen still present in accessory cones (arrow) (periodic acid-Schiff hematoxylin stain); 4: 4 days after iodoacetate, rods and cones no longer distinguishable, profound disorganization of granular layers, and migration of pigment cells; 5: 6 days after iodoacetate, only pigment cell layer recognizable. (From Rabinovitch *et al.*, 1954.)

Malate and pyruvate can partially protect rats against the lethal effects of iodoacetate, but giving malate with iodoacetate produces a much more severe retinal degeneration than with iodoacetate alone, malate by itself having no effect (Graymore and Tansley, 1959 a). The inhibition of glycolysis in the retina *in vivo* by iodoacetate does not appear to be altered by giving malate (Graymore and Tansley, 1959 b). The mechanism of this
potentiation is unknown; the effect of malate may not be on the retina but perhaps through some systemic changes, such as in the blood pH, altering the rate of penetration of iodoacetate into the retina. Some protective effect is observed in the retina by cysteine given intravenously 90 min before and after iodoacetate at a dose of 300 mg/kg (Sorsby and Harding, 1960), but inspection of the data shows the protection to be quite weak, as would be expected with this schedule.

Lens

Cataract has generally been explained as due to an opacity resulting from a defect in the carbohydrate metabolism of the lens. For this reason Nordmann et al. (1954) applied various inhibitors to perfused calf lenses for fairly long periods of time and then examined the tissue for metabolic and opacity changes. Iodoacetate causes clouding of the lens more rapidly than the other inhibitors (within 2 hr), but was used at the unfortunately high concentration of 21.6 mM. Even at this concentration the lens lactate and ATP levels are not markedly reduced. Cibis and Noell (1955) chose the more obvious approach and injected iodoacetate intravenously into rabbits (20 mg/kg) in two doses 6-8 hr apart. The animals were examined 2 months later. The development of opacification accompanied by numerous vacuoles, granules, and a subcapsular iridescence was observed and, histologically, a swelling of the lens fibers, vacuolization of the posterior cortex, and deformation and displacement of the epithelial cells. Lenticular changes were noted in about 65% of the rabbits and were said to resemble the effects of X-radiation (Cibis et al., 1957). Iodoacetate at 5 mM inhibits the penetration of glucose into the lens but probably less than 35% (Müller, 1939). The ion and water pumps in lens are apparently inhibited by iodoacetate, since K+ is lost and Na+ and water gained, although 6 hr in 25 mM iodoacetate could scarcely be used to interpret the effects in vivo (Harris and Gehrsitz, 1951). However, Kinoshita et al. (1961) found the transport systems to be quite sensitive to iodoacetate, since even 0.03 mM exerts a marked inhibition of K+ uptake and Na+ extrusion over 20 hr. The uptake of phosphate by rabbit lens is 57% inhibited by 4.8 mM iodoacetate, and there is a substantial loss of ATP (Müller and Kleifeld, 1953). Whether these actions relate in any way to the development of lens opacity is not known.

Other Eye Tissues

Intravenous injections of 20 mg/kg iodoacetate were noted by Cibis et al. (1957) to inhibit aqueous fluid secretion by the ciliary processes, since there is a fall in the intraocular pressure by 2 days, reaching a minimum in 3 days, and recovering in 5–7 days. The actual rate of aqueous flow is depressed 27% at 2 days and 58% at 3 days by two spaced injections of iodoacetate. The glycolytic rate in the ciliary processes is quite high and was esti-
mated to attain a $Q_{\text{CO}_2}^N$ of around 20 by De Roetth (1954). This is inhibited 75% aerobically and 67% anaerobically by 1 mM iodoacetate, so that either the inhibitor does not penetrate well or the glycolytic system is relatively insensitive. Accumulation of iodide by the rabbit ciliary body–iris preparation is strongly inhibited by iodoacetate, 50% reduction of the tissue/medium ratio being produced by 0.2 mM (Becker, 1961). Iodoacetate also causes rather marked hydration of the cornea (Philpot, 1955; Harris, 1957). It is thus clear that the retina is not the only sensitive ocular tissue, and in animals treated with the higher dosages of iodoacetate many changes of varying degrees have been noted in lens, cornea, and ciliary body. However, there is no doubt that the retina is the most sensitive to iodoacetate.

**EFFECTS ON MISCELLANEOUS CELL AND TISSUE FUNCTIONS**

A few examples of the actions of iodoacetate on relatively little studied functions, for the most part some form of protoplasmic motility, will be briefly presented, not so much for the information they contain but that they may stimulate more work in some of these fields.

**Salivary Secretion**

Stimulation through the nerve produces a rise in secretion and an output of lactate in the isolated perfused submaxillary gland of the cat (Ferrari and Höber, 1933). It can be calculated that 0.054 mM iodoacetate reduces secretion and lactate formation 81%, and simultaneously the Cl⁻ concentration rises markedly. Acetylcholine similarly stimulates the cat salivary gland (increased secretion, lactate, and respiration) and iodoacetate at low concentrations (0.027 mM) depresses this response to acetylcholine (Druckrey and Loch, 1943). Somewhat higher concentrations (0.13 mM) depress the acetylcholine response completely, and respiration slowly and partially; the highest concentration used (0.54 mM) reduces the respiration to zero and kills the tissue. Here, in contrast to muscle, the depression of lactate formation (measured as CO₂ produced) runs more or less parallel with the depression of the response to acetylcholine, so that it was concluded that this response depends on lactate production. If secretion is indeed closely related to glycolysis, it would be interesting.

**Phagocytosis**

Leucocytes have a rather high capacity for anaerobic glycolysis ($Q_{\text{CO}_2}^N$ is around 20) although respiration and aerobic glycolysis are normal relative to other tissues, so that the absolute Pasteur effect is reasonably high.
Furthermore, leucocytic motility and phagocytosis can proceed quite well under anoxic conditions or in the presence of high concentrations of cyanide. One might predict leucocytes to be sensitive to glycolytic inhibitors, at least under anaerobic conditions. Such was first shown by Ferrari and Höber (1933), iodoacetate at 0.1 mM strongly inhibiting the ability of leucocytes to pick up carmine or charcoal particles, and this was confirmed by Köhler et al. (1951), even 0.027 mM iodoacetate significantly depressing phagocytosis. An even greater sensitivity was noted by Siess (1953), the phagocytosis of starch granules by guinea pig polymorphonuclear leucocytes being inhibited 50% by 0.005 mM iodoacetate. Initiation of phagocytosis is accompanied by an elevation of the respiration and lactate formation, whether glucose is present or not, so that apparently phagocytosis initiates an activity type of metabolism (Becker et al., 1958). Evidence that active glycolysis is necessary for phagocytosis was obtained by Sbarra and Karnovsky (1959), but there is an increase in the pentose-P pathway during phagocytic activity also, whereas oxidative phosphorylation is not important. The metabolic effects of iodoacetate are summarized in Table 1-37. Iyer et al. (1961) also concur that the energy for phagocytosis apparently comes from glycolysis, and confirmed the stimulation of resting respiration by 0.1 mM iodoacetate, and the depression of the activity respiration. Although the resting respiration is augmented, the formation of C\textsuperscript{14}O\textsubscript{2} from glucose-1-C\textsuperscript{14} is markedly depressed. On the other hand, pyruvate is able to reverse partially the depression of phagocytosis by 0.2 mM iodoacetate, although the

### Table 1-37

**Effects of Iodoacetate on Glucose Metabolism of Guinea Pig Polymorphonuclear Leucocytes**

<table>
<thead>
<tr>
<th>Property or metabolite</th>
<th>Controls</th>
<th>Iodoacetate (0.1 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resting</td>
<td>Phagocytosing</td>
</tr>
<tr>
<td>Respiration (Q\textsubscript{O\textsubscript{2}})</td>
<td>16.9</td>
<td>43.9</td>
</tr>
<tr>
<td>C\textsuperscript{14}O\textsubscript{2} formed from:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose-1-C\textsuperscript{14}</td>
<td>254</td>
<td>2121</td>
</tr>
<tr>
<td>Glucose-6-C\textsuperscript{14}</td>
<td>55</td>
<td>96</td>
</tr>
<tr>
<td>Ratio of C-1/C-6</td>
<td>4.6</td>
<td>22.7</td>
</tr>
<tr>
<td>Lactate (\textmu g)</td>
<td>586</td>
<td>496</td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>Normal</td>
<td>Minimal</td>
</tr>
</tbody>
</table>

respiration is not simultaneously elevated much (Sbarra and Shirley, 1963). It is even more surprising that pyruvate reverses to about the same degree anaerobically, so its effects presumably are not mediated through the cycle.

**Streaming of Myxomycete Plasmodium**

This type of motility seems to be much more sensitive to inhibitors of oxidations (e.g., cyanide or azide) than to iodoacetate. Allen and Price (1950) found that low concentrations of iodoacetate stimulate the respiration somewhat, 2 mM has no effect, and 5 mM inhibits 65%, but the flow is not stopped until the plasmodium disintegrates. The results of Ohta (1954) are quite similar, except that 1 mM iodoacetate was found to inhibit slightly. More recent work has emphasized that motility and motive force are more sensitive to iodoacetate than is the respiration. In *Physarum polycephalum* a concentration of 1 mM has no effect on the respiration but motility is reduced significantly (Kamiya et al., 1957). Iodoacetate at 2 mM almost abolishes motility and the protoplasmic viscosity increases so much that measurements of motive force are impossible after 30 min. It has often been assumed that plasmodial movement is dependent on ATP, but Hatano and Takeuchi (1960) reported that iodoacetate at 0.67 mM, which reduces the motive force, has no significant effects on ATP or other phosphate fractions, and that 1 mM seems to elevate the ATP level. The ATPase activity is not altered by iodoacetate.

**Streaming of Avena Coleoptile Protoplast**

The protoplasmic streaming in the epidermal cells of the coleoptile is stimulated by 0.001 mM iodoacetate and relatively unaffected at 0.05 mM unless the streaming is accelerated by auxin, in which case iodoacetate prevents this stimulation (Sweeney and Thimann, 1942). Even up to 0.2 mM there is no reduction of the auxin-accelerated streaming below the levels in the absence of auxin. Malate is able to overcome partly the effect of iodoacetate on the auxin-induced streaming. This is a good example of how an inhibitor can rather selectively depress an augmented activity without significantly affecting the basal level of function, and such behavior leads one to suspect that the pattern of metabolism is altered by the stimulation. Since 0.65 mM iodoacetate prevents growth completely, it is possible that the interference with auxin-induced streaming is involved.

**Spermatozoal Motility**

Flagellar movement in general seems to depend on ATP and the usual sources of ATP, but in bull spermatozoa the situation is not so clear. Lardy and Phillips (1941) found that iodoacetate at concentrations of 0.25 mM
and above inhibits anaerobic glycolysis completely, and yet motility persists. However, 0.5 mM iodoacetate reduces the motility aerobically during the first 30 min and almost abolishes it during the next hour. Thus one might be more inclined to conclude that motility is not immediately dependent on glycolysis or oxidations, and that the stores of creatine-P and ATP are sufficient to maintain activity for some time, and in this respect the situation is not essentially different from muscle. Besides, 0.5 mM iodoacetate inhibits glucose utilization aerobically only 31% during the first hour (Lardy and Phillips, 1943a). The incorporation of C\textsuperscript{14}-labeled amino acids into spermatozoa is not significantly reduced by 5 mM iodoacetate aerobically, but anaerobically it is inhibited quite well, whereas motility is abolished under both conditions (Bhargava et al., 1959). There seems to be no correlation between motility and amino acid incorporation. Monroy (1948) noted that the spermatozoa of Pomatoceiros triqueter lose much of their fertilizing ability in 1 mM iodoacetate but the motility is not impaired. The spermatozoa cannot adhere to the vitelline membrane, and it was suggested that the lytic factor in the head may be an SH enzyme of cathepsin type.

**Ciliary Motility**

The injection of 400 mg/kg bromoacetate into frogs leads to skeletal muscle rigor, then cessation of the heart beat, but only later is there evidence of depression of pharyngeal ciliary activity (Wertheimer, 1932). It was stated that the cilia pass into a state of rigor, but this is admittedly difficult to determine. Isolated pharyngeal cilia preparations show some depression by 0.15-0.21 mM bromoacetate after 5 hr and a sporadic beating within 2 hr in 0.31 mM bromoacetate. A concentration of 1.2 mM stops the cilia in 1 hr. Pyruvate and lactate counteract these effects. Paramecium motility is not affected by bromoacetate, even in combination with cyanide. The cilia of the excised gills of the mussel Mytilus edulis show no alteration of motility with 1 mM iodoacetate in sea water (pH of 8), although phosphate uptake is inhibited around 20%, and it was postulated that the lack of action may be due to the failure to penetrate at this pH, any effects observed being due to direct action on the proteins at the cell surfaces (Ronkin, 1950).

**Vesicant Action of Iodoacetate**

The concept that vesication is related to the blocking of SH groups arose during World War II in connection with the interest in war gases. Bacq and Goffart (1940) pointed out that bromoacetic and iodoacetic acids are quite potent vesicants when applied to the skin, the lesions being indistinguishable from those produced by the war gases and common vesicants such as allyl isothiocyanate, chloroacetophenone, chloropicrin, and others. A lag period of several hours was noted. Bacq (1941) claimed that vesicants in
general inhibit glycolysis and often give the Lundsgaard effect on skeletal muscle. Peters and Wakelin (1949) were inclined to relate the inhibition to pyruvate oxidase. Iodoacetamide is a potent inhibitor of this enzyme ($pK_{50} = 4.3$) but no data on its vesicant action were available, except that one of the authors produced a large erythematous patch by subdermal injection of 1.5 mg. Flesch and Goldstone (1950) had experimentally shown iodoacetic acid to be a primary irritant, and that 10 min contact is sufficient to cause a painful bullous lesion, followed by superficial epidermal necrosis. Two cases of dermatitis due to iodoacetic acid were reported by Marcus and Frerichs (1950). A chemist allowed some iodoacetic acid solution to trickle over his hands and immediately washed it off; in 5 hr the fingers burned and a bullous eruption occurred and in 2 days there were large bullae on the palmar surfaces of all fingers, and on palms and wrists. Healing required almost a month. The other case presented similar findings, including erythema, vesicles, and depigmentation, and also required a long time to heal. Application of 0.3 ml 5–10% solutions of iodoacetic acid to depilated areas of guinea pig skin leads to vesication. erythema, itching, separation of the epidermis from the corium, and eventual healing in several days (Flesch et al., 1952). One may note that despite much talk about the vesicant actions of iodoacetate and its relation to SH reaction, very few experiments have been done. In most cases no controls were run; for example, it might be interesting to see what 5–10% acetic acid would do to depilated guinea pig skin. And one must always ask whether at least some of the effects noted are due to a nonspecific acid effect. In connection with the vesicant action of iodoacetate when applied locally, it is interesting that edema produced subcutaneously by injections of formalin or dextran is quite well prevented in rats by injections of iodoacetate (Stenger, 1959). However, subcutaneous injections of iodoacetate cause swelling.

**EFFECTS OBSERVED IN THE WHOLE ANIMAL**

The toxic effects observed on administration of near-lethal doses of bromoacetate and iodoacetate are mainly referable, during the early stages of poisoning, to the central nervous and cardiovascular systems. The original observations of Steinauer (1874) and Pohl (1888) on the sequence of toxic reactions in frogs and rabbits given bromoacetate have been presented (pages 1-2).

**Distribution in Animals**

The effects upon various tissues in an animal may depend to some extent on the distribution of an inhibitor in the tissues. Thomassen and Leicester (1951) studied the distribution of iodoacetate-I$^{131}$ in rats after intraperitoneal injection of a toxic dose in an investigation of the effects of iodoacetate on
dental caries (Table 1-38). Since iodoacetate-I\textsuperscript{131} may release some inorganic I\textsuperscript{131} into the tissues, a comparable study was made using iodide-I\textsuperscript{131}. The maximal uptake occurred around 3 hr and the analyses were done at this time. Iodide is picked up selectively only by the thyroid and the concentrations in the other tissues are low. Iodoacetate concentrations are

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Iodoacetate-I\textsuperscript{131}</th>
<th>Iodide-I\textsuperscript{131}</th>
<th>Ratio of IA to I\textsuperscript{-}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thyroid</td>
<td>8.304</td>
<td>152.45</td>
<td>0.055</td>
</tr>
<tr>
<td>Blood</td>
<td>1.201</td>
<td>0.698</td>
<td>1.72</td>
</tr>
<tr>
<td>Saliva</td>
<td>0.948</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Adrenals</td>
<td>0.648</td>
<td>0.226</td>
<td>2.86</td>
</tr>
<tr>
<td>Molars</td>
<td>0.505</td>
<td>0.211</td>
<td>2.39</td>
</tr>
<tr>
<td>Liver</td>
<td>0.467</td>
<td>0.255</td>
<td>1.87</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.402</td>
<td>0.266</td>
<td>1.51</td>
</tr>
<tr>
<td>Incisors</td>
<td>0.397</td>
<td>0.184</td>
<td>2.16</td>
</tr>
<tr>
<td>Salivary gland</td>
<td>0.358</td>
<td>0.250</td>
<td>1.43</td>
</tr>
<tr>
<td>Bone</td>
<td>0.285</td>
<td>0.187</td>
<td>1.52</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.159</td>
<td>0.111</td>
<td>1.43</td>
</tr>
</tbody>
</table>

\*Iodoacetate injected intraperitoneally at a dose of 50 mg/kg in rats and tissues analyzed at 3 hr. The figures give the per cent of the administered dose of labeled substance per gram fresh weight of tissue. (From Thomassen and Leicester, 1951.)

about twice those of iodide in most tissues. The high thyroid level after administration of iodoacetate is probably due to the release of a small amount of labeled iodide, which is avidly accumulated. This may also explain the somewhat greater concentration in saliva. There is no evidence for appreciable selective uptake of iodoacetate by any tissue. It is interesting that skeletal muscle has a relatively low concentration of iodoacetate, which might be anticipated on the basis of the general anionic impermeability of muscle. It is unfortunate that the brain was not analyzed, but it is likely that the iodoacetate level would have been quite low. The retention in the tissues seems to be rather long, but it is not known if this represents iodoacetate or iodide.

When iodoacetamide-I\textsuperscript{131} is injected intraperitoneally into mice, the blood level rises rather rapidly and falls rapidly during the first hour, being essentially gone by 7 hr (Friedman and Rutenberg, 1950 b). The initial rapid fall
may be due to uptake by the tissues and the later plateau to labeled iodide released from the iodoacetamide. The liver contains about 3% of the blood level originally and this disappears by 7 hr. Chronic feeding of bromoacetate to pigs (around 50 mg/kg for 39 days with a total dose of 31 g) leads eventually to death, at which time the tissues were analyzed for organic Br by Dalgaard-Mikkelsen et al. (1955). The results are shown in the accompanying tabulation. Lower dosage (15–20 mg/kg) over 425–460 days gave roughly 

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Organic Br (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>540</td>
</tr>
<tr>
<td>Liver</td>
<td>410</td>
</tr>
<tr>
<td>Kidney</td>
<td>345</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>110</td>
</tr>
</tbody>
</table>

the same or somewhat higher levels. One problem is the form of this organic Br. Bromoacetate reacts with SH groups in the tissues and bromide is released, so that reacted bromoacetate would not be expected to contribute directly to these values. Incorporation of bromide into organic compounds in significant amounts seems unlikely.

Acute Toxic Reactions

The first adequate description of iodoacetate poisoning was made by Hall and Field (1932) at Stanford. They injected unanesthetized starved rats intraperitoneally with varying doses of iodoacetate and found, as many others have confirmed, that the chronological sequence of reactions to iodoacetate depends markedly on the dose; i.e., not only the survival time but also the pattern of the toxic response is altered by dosage level. They delineated two response patterns, one from high doses where death is rapid and one from lower doses where the animals survive for several hours. Animals receiving 120 mg/kg or over die within 20 min and symptoms begin in 8 min. The effects may be summarized in the order in which they occur.

(1) The first sign is a marked hypoactivity, punctuated by attacks of hyperactivity (running, climbing, biting), which soon becomes ataxic and occasionally takes the form of circus movements.

(2) Distinct hyperpnea which progresses to inspiratory dyspnea.

(3) Animals become prostrate on one side and cannot right themselves, the hind legs paralyzed in abduction.

(4) Occasional mild tremors or clonic convulsions appear and the animal is hyperexcitable.
(5) Sudden violent tonic convulsions of the whole body occur, lasting about 10 sec, leaving the animal in a state of rigor (opisthotonus, upper extremities flexed and lower extremities extended), and respiration ceases with a few gasping movements.

The heart continues beating and probably would have continued much longer if asphyxia had not been induced by respiratory failure. Animals receiving around 70–90 mg/kg survive longer, show many of the above effects, but exhibit a somewhat different terminal picture. Clonic convulsions, often of a rolling type, occur in repeated attacks over a long period of time rather than a sudden tonic phase, and do not cause death. The respiratory rate progressively slows and death occurs without terminal convulsions, rigor not appearing until 15 min after death. Finally, animals receiving 30–50 mg/kg, in which survival is over 24 hr, show maximal hypoactivity at 4 hr and no signs of convulsions. It was believed that the earliest effects of iodoacetate are on the central nervous system and only later are the muscles or circulation adversely affected. Neuss (1931) found very similar reactions in mice and rabbits to intravenous injections of bromoacetate, death occurring from respiratory failure following convulsions. However, in anesthetized rabbits in which small amounts of bromoacetate are injected over a period of time, circulatory collapse can occur and this is the cause of death. It is probable that anesthesia protects the central nervous system by reducing its activity, thus helping to maintain levels of creatine-P and ATP, and also counteracts hyperactivity and convulsions. Indeed, Neuss found urethane to raise the lethal dose of bromoacetate, as did Haarmann (1932). Intravenous bromoacetate (2–8 mg/kg) produces varying degrees of ataxia in dogs; at the higher doses the ataxia is prolonged to several days (Andersen et al., 1955). As the dosage is increased to 16–24 mg/kg, early periods of apathy are superimposed on the ataxia, with a greater tendency to muscular rigidity. The mechanism by which iodoacetate produces the central nervous system effects is obscure. Possibly the convulsions are due to a fall in the membrane potentials in certain areas which discharge spontaneously, or even to the depression of certain inhibitory areas. Convulsive phases are often observed in poisoning with other metabolic depressants (e.g. cyanide), or in asphyxia or hypoglycemia. There is no evidence that the central effects are related to changes in the blood chemistry.

Hyperglycemia and Other Changes in the Blood

Predictions of the response of blood glucose levels to iodoacetate are difficult because there are not only the more or less direct effects on tissue metabolism, but perhaps many secondary factors. The experimental results have been by no means uniform. A rise in the blood glucose in rabbits injected with 40–50 mg/kg bromoacetate was described by Neuss (1931) (see ac-
companying tabulation). This would harmonize with the results of Haar- 
mann (1932), who showed that bromoacetate has no effect on glycogenolysis, 
but reduces glucose utilization; hyperglycemia on this basis would be due 

to a reduced rate of glucose utilization. Hikiji (1932) showed that there is 
a rather marked loss of muscle glycogen following injection of 20–40 mg/kg 
bromoacetate. It is possible that some of the blood glucose originates from 
this glycogen. Irving (1934) pointed out that hyperglycemia is seen only 
when lethal or near-lethal doses of iodoacetate are given, although the 
mortality does not depend on the hyperglycemia, since insulin will counter-
act the hyperglycemia without protecting the animal. Subcutaneous doses 
of iodoacetate (80 mg/kg) in starved rabbits lead to a rise in blood glucose 
from 125 mg% to 210 mg% in 40 min. Irving tended to believe that the 
reduction in the tissue utilization of glucose is the principal factor in caus-
ing the hyperglycemia. Definite hyperglycemia was also reported by Hand-
ler (1945), who found 180% increase of blood glucose after 71 mg/kg iodo-
acetate injected into rabbits, and El Hawary (1955), who found a 2-fold 
increase after 75 mg/kg iodoacetate intraperitoneally into rats. On the 
other hand, hypoglycemic responses have also been encountered. Rex-Kiss 
and Zsadon (1939) observed only decreases of 10–35% in the blood glucose 
in dogs given 10–25 mg/kg iodoacetate intravenously, and Hultquist (1958) 
reported around 20% depressions of blood glucose from 50 mg/kg iodoac-
etate or 30 mg/kg iodoacetamide subcutaneously in rats, no hyperglycemia 
being noted at any time. The last two reports deal with rather low doses of 
iodoacetate, while those who found hyperglycemia generally used lethal or 
near-lethal doses. Unfortunately no complete study of the dosage range in 
any animal has been made, so it is impossible to relate these discrepant 
results entirely to dosage, as many other factors may be involved.

Blood glucose level depends on the balance between the rates of a few 
transport processes, mainly the absorption from the intestine, the excretion 
by the kidney, and the transfers in and out of the other tissues (Fig. 1-26). 
The effects of iodoacetate may well depend on whether the animals have 
been starved or not, since glucose absorption through the intestine is inhib-
ited by iodoacetate (Wilbrandt and Laszt, 1933; Klinghoffer, 1938; Öhnell 
and Höber, 1939; Laszt, 1939). If glucose absorption is an important factor
in controlling the blood glucose level, iodoacetate by this effect will tend to cause a hypoglycemic reaction. If the animals have been starved and no appreciable glucose absorption is occurring, there is probably a mobilization of glucose from the liver (and perhaps other tissues) to supply the needs of the body. There is no evidence that iodoacetate at the doses used significantly alters the renal excretion of glucose, so we can eliminate this factor (see page 192). Iodoacetate at fairly low doses (15 mg/kg) effectively

![Diagram of liver, gut, blood, kidney, and other tissues in normal and starved conditions.]

Fig. 1-26. Schematic representations of how blood glucose may be altered. The lengths of the arrows indicate roughly the amounts of glucose being transferred between the compartments. See text for further explanation.

depresses the accumulation of liver glycogen induced by either insulin or epinephrine (Goldblatt, 1933), but high doses (60 mg/kg) do not interfere with liver glycogenolysis (Noltie, 1935). Hultquist (1958) reasoned that if alloxan alters blood glucose by an action on pancreatic insulin release, iodoacetate might also, and found some nuclear enlargement of the β-cells, indicating overactivity, following iodoacetate. Falkmer (1962) observed a nonspecific lowering of islet GSH after the LD_{50} dose of iodoacetate in Cottus scorpius, but whether this indicates that any of the blood glucose changes are mediated through the pancreas is not known. Another factor of possible importance, especially with high doses of iodoacetate, was emphasized by Irving (1934), namely, sympathetic stimulation, perhaps originating in the midbrain inasmuch as the hyperglycemic response to iodoacet-
tate is partially blocked by ergotoxine. One might temporarily suggest the changes shown in Fig. 1-26, pending further experimental evidence, as showing the major effects of low and high doses of iodoacetate. At low doses, tissue utilization of glucose is not appreciably depressed directly and may be increased somewhat due to release of insulin whereas, at lethal dosage levels, tissue utilization of glucose is mainly suppressed and sympathetic stimulation is superimposed. Other factors can well be imagined; e.g., the fall in blood pressure usually seen after iodoacetate may also contribute to sympathetic and adrenal activation.

It must not be assumed that all the effects of toxic doses of iodoacetate are attributable entirely to a selective block of the EM pathway. There is evidence that other pathways must be affected. For example, blood keto acids (pyruvate and α-ketoglutarate) rise to about the same degree as blood glucose after high doses of iodoacetate (El Hawary, 1955; Handler, 1945), and acetone bodies (acetone and acetoacetate) may be elevated also.

**Chronic Toxicity and Tissue Damage**

The marked effects of iodoacetate on the retina prompt us to inquire whether other tissues may be especially sensitive to this inhibitor, due to either a predominant glycolytic activity or a functional dependence on glycolysis, or a particular function (such as cell proliferation) which involves SH groups other than those of 3-PGDH. Karli (1952) in his study of retinal degeneration examined animals several days after the administration of iodoacetate to determine if the retinal damage is indeed selective, and discovered marked degenerative changes in the testes. The formation of spermatozoa was completely inhibited and the cells concerned with spermatogenesis were damaged; the epididymal canal contained spermatids and spermatocytes in various stages of degeneration. Certainly testis as a whole does not have an unusually high glycolytic activity, but here one is concerned with only a certain type of cell which may have quite different metabolic characteristics than the bulk of the cells. Many types of proliferating cells seem to depend on the glycolytic pathway, but in addition have a reasonably high energy requirement (see page 260). It has been demonstrated that invertebrate spermatogenesis in vitro is very sensitive to iodoacetate, 50% inhibition being given by 0.04 mM (Schneiderman et al., 1953). Injections daily of 5 mg bromoacetate in rats lead to marked atrophy of the seminal vesicles in a few days (Schiller, 1935). A cessation of the estrus cycle in female rats occurs. Hematopoietic depression might also be anticipated, and Laszt and Verzár (1936) observed a 28% reduction in erythrocyte count and a comparable fall in hemoglobin in young rats fed 1–2 mg iodoacetate per day. The skin has a relatively high proliferation rate, and it was noted that a dermatitis appeared between 8 and 14 days, with subsequent loss of hair.
The intestinal mucosa is often damaged by substances which depress cell growth and mitosis (e.g., the antitumor drugs), so it is not surprising that effects from iodoacetate administration have been observed. Laszt and Verzár (1936) found a striking enlargement of the whole gastrointestinal tract in poisoned rats at 34 days, both stomach and intestine being essentially doubled in weight. The injection of high doses (60–200 mg/kg) of iodoacetate in rats causes pyloric spasm, hemorrhagic enteritis, and gross pathological changes in the intestine (Klinghoffer, 1938). The intestine shows spastic contractions, increased mucus secretion, and microscopic damage to the epithelium and villi (Öhnell and Höber, 1939). Giving rats water which is 2.7–5.4 mM in iodoacetamide leads to gastritis and gastric ulcers, although iodoacetate does not do this, perhaps due to permeability differences (one might expect the low gastric pH to favor iodoacetate penetration, however). Another effect of iodoacetate on the intestine may or may not be related to this damage: this is the inhibition of fat absorption, which is very marked (Verzár and Laszt, 1934) and may lead to steatorrhea (Laszt and Verzár, 1935). Laszt and Verzár (1936) postulated a relationship between this state and coeliac disease (intestinal infantilism, *Gee-Hertersche Krankheit*), since the symptoms are similar and both are relieved by the administration of yeast, but whether this involves adrenal dysfunction, abnormalities in intestinal absorption, vitamin deficiencies, or something else, is not known.

Renal damage may be caused by higher doses of iodoacetate; this may be due not to a special sensitivity but to exposure to a higher concentration of inhibitor than most tissues. The most obvious effect is a necrosis of the convoluted tubules (Stevenson and White, 1940), characterized by cellular fragility, occlusion of the tubules, vacuolization of the tubular cells, and dilatation of the vessels (Becker and Rieken, 1934). Other effects observed but not studied extensively are the osteoporosis, which could well be due to inadequate intestinal absorption of calcium (Laszt and Verzár, 1935), but there is evidence that deposition of calcium may also be disturbed (Verzár and Laskowski, 1937); adrenal cortical hypertrophy (Laszt and Verzár, 1936) with simultaneous reduction in hormone levels (Giroud et al., 1941); and a generalized edema of animals (Hikiji, 1932). The total excretion of Na⁺ and K⁺ by rats is decreased by 25 mg/kg iodoacetate intraperitoneally; during the first day the Na⁺ excretion drops from 36.5 to 9 mg/kg body weight, and the K⁺ from 146 to 116 mg/kg body weight, but on succeeding days the excretion returns to normal (Boccacci and Quintiliani, 1960). This is due mainly to the intestinal stasis and reduction in fecal output.

**Toxic and Lethal Doses**

A collection of data on toxic and lethal doses is given in Table 1-39, from which no obvious species differences are evident. The LD₅₀ is usually 50–80 mg/kg, corresponding to 0.27–0.4 millimole/kg of iodoacetate. Bromo-


### Table 1-39

**Toxic and Lethal Doses of Iodoacetate**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Route</th>
<th>Dose&lt;sup&gt;a&lt;/sup&gt; (mg/kg)</th>
<th>Effect</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>SC</td>
<td>75</td>
<td>LD₅₀</td>
<td>Fitzgerald (1955)</td>
</tr>
<tr>
<td></td>
<td>Oral</td>
<td>83</td>
<td>LD₅₀</td>
<td>Morrison (1946)</td>
</tr>
<tr>
<td></td>
<td>IP</td>
<td>55</td>
<td>LD₅₀</td>
<td>Kramer &lt;i&gt;et al.&lt;/i&gt; (1963)</td>
</tr>
<tr>
<td>Rat</td>
<td>IV</td>
<td>40</td>
<td>Tolerated</td>
<td>Smythe and Reiner (1933)</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>60</td>
<td>LD₅₀</td>
<td>Graymore and Tansley (1959 a)</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>100</td>
<td>Most die</td>
<td>Klinghoffer (1938)</td>
</tr>
<tr>
<td></td>
<td>IP</td>
<td>30–50</td>
<td>MLD</td>
<td>Hall and Field (1932)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>70</td>
<td>ED₅₀ (150 min)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>90</td>
<td>ED₅₀ (25 min)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IP</td>
<td>50</td>
<td>Toxic</td>
<td>Thomassen and Leicester (1951)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>IV</td>
<td>5–15</td>
<td>Nonlethal</td>
<td>Goldblatt (1933)</td>
</tr>
<tr>
<td></td>
<td>SC</td>
<td>60–70</td>
<td>Lethal in 15 min</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>80</td>
<td>MLD</td>
<td>Irving (1934)</td>
</tr>
<tr>
<td>Dog</td>
<td>IV</td>
<td>50</td>
<td>LD₅₀</td>
<td>Haldi (1932)</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>33</td>
<td>Some die</td>
<td>Henderson and Greenberg (1934)</td>
</tr>
<tr>
<td></td>
<td>IV</td>
<td>40</td>
<td>All die</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IP</td>
<td>20</td>
<td>Nonlethal</td>
<td>Rex-Kiss and Zsadon (1939)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>45</td>
<td>LD₅₀</td>
<td>Kramer &lt;i&gt;et al.&lt;/i&gt; (1963)</td>
</tr>
</tbody>
</table>

<sup>a</sup> In most cases the dosage refers to iodoacetic acid rather than to a salt, but in some it is impossible to determine exactly the weight basis.

acetate is somewhat less toxic. The resistance to iodoacetate apparently varies with the age, since newborn mice can survive as much as 500 mg/kg subcutaneously, whereas the adult LD₅₀ is 75 mg/kg (Fitzgerald, 1955). Since iodoacetate acts essentially irreversibly it can exert its effects for a long time, so that even small doses may be lethal after a prolonged interval. Hall and Field (1932) studied the relationship between intraperitoneal dose in rats and survival time, and found that at doses of 130 mg/kg or above the time for 50% survival is 10 min or somewhat less; most of this delay is due to the time for absorption. When the dose falls below 90 mg/kg, the survival time become rapidly longer, so that at 70 mg/kg it is 150 min, and at 50 mg/kg all rats survive at least 24 hr.
EFFECTS ON MITOSIS, GROWTH, AND DIFFERENTIATION

Interference with proliferation and growth of cells by inhibitors is often attributable to the inhibition of the syntheses of certain necessary cell components. Iodoacetate is able to depress the formation of most of the basic macromolecules and substances involved in the cell structure; these effects have been discussed in the sections on metabolism of proteins (page 147), polysaccharides (page 133), photosynthesis (page 157), lipids (page 144), and porphyrins (page 155). We shall only add here a few observations which seem pertinent to the problems of growth. In most cases the inhibition of the syntheses of complex molecules is nonspecific in the sense that it is due simply to a reduction in the energy available for assemblage, and very little is known about the possibilities of specific interference in the more terminal pathways.

Iodoacetate depresses protein synthesis quite potently but the exact location of the site, or sites, of attack has not been determined. In addition to the investigations previously presented, we may note that the following are markedly inhibited: the incorporation of acetate-1-C\(^{14}\) into tumor proteins (van Vals and Emmelot, 1957), the formation of the adaptive enzyme for the oxidation of benzoate in Mycobacterium \textit{lacticola} (Fitzgerald \textit{et al.}, 1949), the biosynthesis of proteinase in \textit{Streptococcus liquefaciens} (Rabin and Zimmerman, 1956), and the incorporation of a variety of amines into the proteins of guinea pig liver (Clarke \textit{et al.}, 1959). The studies of Ogata and his associates (1956, 1958) are particularly interesting. They followed the incorporation of glycine-C\(^{14}\) into the antibody to ovalbumin and various other proteins in rabbit lymph nodes. The formation of antibody, nucleoprotein, mitochondrial protein, and microsomal protein is reduced 70–85% by 2 mM iodoacetate in cell suspensions, and comparable inhibitions are produced in homogenates. The incorporation of glycine-C\(^{14}\) into the proteins of slices of regenerating liver is more strongly inhibited, essentially a complete block being established by 1 mM iodoacetate. There is also evidence that iodoacetate can interfere with the biosynthesis of purines, as in ascites tumor cells (Henderson and LePage, 1959), or of nucleic acids; as in brain slices (Findlay \textit{et al.}, 1953) and in spleen, intestine, and thymus (Quintiliani \textit{et al.}, 1961). Glycogen synthesis is almost invariably depressed by iodoacetate, but other polysaccharides may be included, for example that of pneumococcal capsules, the formation of which is strongly inhibited by 0.1 mM iodoacetate (Bernheimer, 1953). A full realization of such biosynthetic inhibitions must form one basis for the interpretation of the studies on growth and differentiation.

Mitosis

The effects on mitosis and cleavage associated with plant growth, embryogenesis, tumors, and proliferation of microorganisms will be taken up
in the succeeding sections, and only the specific studies on mitosis will be considered here. The growth of chick embryo fibroblasts in culture was found by Krontowski et al. (1932 b) to be completely suppressed by 0.05 m\(M\) iodoacetate, glucose utilization being simultaneously reduced 93\%. This concentration also prevents growth of chick embryo brain cultures, while 0.02 m\(M\) iodoacetate causes fusion of the chromatin in dividing cells to form a pycnotic mass (O’Connor, 1950 a). Certainly iodoacetate is one of the most potent antimitotic agents among the common inhibitors. Harris (1956) found 1 m\(M\) iodoacetamide to cause immediate disappearance of all rat heart connective tissue cells in culture, but did not use lower concentrations. Mouse epidermal fragments in glucose medium show cells in various stages of mitosis; iodoacetate at 0.1 m\(M\) completely blocks initiation of new mitoses within 2 hr, and by 4 hr no cells in mitosis are visible (Bullough and Johnson, 1951). Since mitosis here is dependent on glucose, the inhibition by iodoacetate is not surprising. Gelfant (1960) continued this work and showed that within 4 hr the number of mitoses is depressed 75\% by 0.01 m\(M\) iodoacetate. Various substrates, including pyruvate, lactate, succinate, and \(\alpha\)-ketoglutarate, cannot reverse the action of iodoacetate at 0.05 m\(M\), which almost abolishes mitosis. The survival of skin fragments, as shown by outgrowth in host animals, after being incubated for long periods in physiological medium, is reduced markedly by 0.1 m\(M\) iodoacetate (Medawar, 1947), indicating again the sensitivity of skin to this inhibitor.

More detailed effects of iodoacetate on mitosis in cultures of chick bone were described by A.F.W. Hughes (1950). Of all the inhibitors tested, only fluoride and iodoacetate block pre-prophase activity, i.e., prevent the cells from entering prophase, without affecting interphase cells or the later stages of mitosis. Exposure to 0.05 m\(M\) iodoacetate prevents mitotic entry, and yet 0.51–1.8 m\(M\) if added during metaphase only prolongs this stage somewhat without impairing the spindle and allows normal anaphase and cleavage. It requires 5.4 m\(M\) to block entry into anaphase, and if this concentration is added at anaphase, nuclear reconstruction is disturbed and the nucleoli may be absent. Hughes discussed the possibility that \(\text{SH}\) agents prevent spindle formation by reacting directly with the proteins involved, and there is a good deal of evidence that \(\text{SH}\) groups, other than metabolic, are involved in mitosis (Brachet, 1957). Erythrocytic development in chick embryo blood is also very sensitive to iodoacetate, 0.0067 m\(M\) reducing mitotic cells by 22\% in 90 min (O’Connor, 1952). Chromosomal effects are seen at this concentration and increase at higher concentrations; these consist of swelling and pycnotic changes. No effect on interphase cells can be observed. Since fluoride has quite different effects here, O’Connor postulated that the action of iodoacetate is not due to inhibition of carbohydrate metabolism but presumably to some more direct action on the \(\text{SH}\) groups.
involved in cleavage. On the other hand, Beatty and Beatty (1959) could find no effects of 1 mM iodoacetate on the chromosomes of *Tradescantia* under aerobic conditions, although anaerobically some aberrations are evident.

Mitotic activity can be very significantly affected *in vivo*, as indicated by the previously discussed effects on proliferating tissues, but is especially well characterized in the studies of Cibis *et al.* (1957) on mitosis in lens epithelium of rabbits, in which injections of 40 mg/kg iodoacetate produce very marked and long-lasting effects (Fig. 1-27). The initial brief stimulation is probably real, and such stimulation of mitotic activity by low concentrations of SH reagents has often been observed.

![Figure 1-27. Effects of iodoacetate (40 mg/kg) on the mitotic activity of lens epithelium in rabbits. (From Cibis *et al.*, 1957.)](image)

**Inhibition of Plant Tissue Growth**

Selected growth inhibitions, usually measured with respect to elongation, are given in Table 1-40. These results are not of great quantitative significance since the inhibitions depend so strongly on the pH. However, they show that, when the pH is favorable to penetration, the growth is very sensitive to iodoacetate. A stimulation of the growth at low concentrations has often been noted (Commoner and Thimann, 1941; Hopkins, 1952). This is particularly well seen in the biphasic curves for *Avena* coleoptiles, where stimulation by as much as 50% is observed (Thimann and Bonner, 1948). Depending on the pH, the concentrations of iodoacetate that stimulate are usually in the range 0.001–0.01 mM. No explanation of this stimulation has been made or is evident. A slight depression of the EM
pathway seems unlikely as the cause, so that one must assume an action elsewhere. Perhaps this is directly on the plasma membrane, altering permeability or auxin transport, or there is in some way a sensitization to auxin. If this is so, one must wonder what role this action plays at higher concentrations.

A relationship between inhibition by iodoacetate and auxin has been emphasized since the earliest work, Howard and McClintock (1940) noting that 3-indoleacetic acid can somewhat overcome the inhibition. However, most workers have remarked that iodoacetate seems to block rather specifically the growth stimulation induced by 3-indoleacetic acid (e.g., Christiansen et al., 1949), having much less effect on basal growth.* Pohl and Ochs (1953) found the effects of iodoacetate to vary with the concentration of 3-indoleacetic acid present, although the actions of the auxin on cress roots seem ab-

* Unfortunately much of the earlier work did not include adequate controls, particularly the effects of iodoacetate in the absence of 3-indoleacetic acid, so that the conclusions were unjustified.
normal. There is undoubtedly some relationship, but it may be doubted if it is simple. For example, in the study of Christiansen (1950) on the growth of pea stems, in which the proper controls were run (see accompanying tabulation), the inhibition of basal growth was 62% and the inhibition of

<table>
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<th></th>
<th>Iodoacetate (0.6 mM)</th>
<th>Elongation</th>
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<tr>
<td>3-Indoleacetate</td>
<td>-</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>7.5</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>50.9</td>
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<tr>
<td></td>
<td>+</td>
<td>25.6</td>
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the auxin effect was 41%. Iodoacetate can interfere in the complexing of 3-indoleacetate with pea root proteins (completely at 0.5 mM) and in the reaction of the auxin-protein complex with coenzyme-A (50% at 5 mM (Siegel and Galston, 1953). The binding of auxin to protein in vivo seems to be an energy-requiring process, and is inhibited by a variety of metabolic depressants. Polar auxin transport in sunflower stems is moderately reduced by iodoacetate (Niedergang-Kamien and Leopold, 1957) and this could certainly play some part in growth inhibition under natural conditions.

Little attempt has been made to determine if the inhibitions observed are indeed related to the block of the EM pathway. However, Commoner and Thimann (1941) found that various cycle substrates can to a large extent overcome the growth suppression produced by 0.05 mM iodoacetate. In decreasing order of effectiveness, the following are active: fumarate, malate, pyruvate, and succinate. Albaum and Eichel (1943) also found succinate and pyruvate to be effective antagonists. It seems that iodoacetate acts mainly on the EM pathway, but the order of potency of these substrates is surprising and is not related to their penetrabilities (i.e., their $pK_a$'s). The odd observation of Thimann and Bonner (1948) that malonate and maleate at 1 mM can overcome iodoacetate inhibition of coleoptile growth is probably to be explained as due to the K$^+$ ions introduced with these anions (Cooil, 1952). It has been commonly observed that iodoacetate in concentrations markedly depressing growth does not inhibit respiration much or at all (Commoner and Thimann, 1941; Albaum and Eichel, 1943; Christiansen et al., 1949). Commoner and Thimann believed there to be a small fraction of the respiration associated with auxin-stimulated growth which is particularly sensitive to iodoacetate, but it is doubtful if such an interpretation can now be put on the results in light of the possible reasons for the insensitivity of respiration to iodoacetate (see page 110). The differential effect can be seen well in corn root tips, where the iodoacetate concentrations for 50% inhibition of elongation and respiration are 0.24 mM
and 2.5 mM, respectively (Zöttl, 1953), and Niedergang-Kamien and Leon- 
pold (1957) observed no respiratory inhibition in sunflower stems with 1 mM 
iodoacetate over 4 hr, although auxin transport is well depressed.

The number of cells per root tip in Cucurbita pepo is increased from 
8.2 \times 10^4 to around 8.7 \times 10^4 by low concentrations (0.004-0.04 mM) of 
iodoacetate, but is decreased to 7.35 \times 10^4 and 6.47 \times 10^4 by 0.41 and 
1 mM, respectively (Hopkins, 1952). The decrease in the number of cells 
by 1 mM iodoacetate (21%) is not as much as the decrease in elongation 
(63%), indicating some changes in cell size or form. Iodoacetate also reduces 
the amount of cell wall deposited by apical pea stems, and this does not 
correlate well with the changes in carbohydrate utilization (Christiansen 
and Thimann, 1950 a). Meiosis in the excised anthers of Lilium henryi is 
inhibited quite strongly as the iodoacetate concentration is raised from 
0.01 mM to 1 mM (Pereira and Linskens, 1963). The only histological study 
of the effects of iodoacetate was made by Torrey (1953) in isolated pea root 
tips. There is acceleration of the maturation of xylem tissue, with absence 
of mitotic cells along the root in the apical meristem. Secondary wall forma-
tion is abnormal, the newly formed protoxylem being thin and having de-
cicient lignification. The maturation of the sieve tube elements in the phloem 
is normal, but again there is less secondary thickening of the walls in the 
mature cells. Finally, iodoacetate at fairly low concentration (0.3 mM) in-
hibits the uptake of water by discs of potato (Hackett and Thimann, 1950) 
and artichoke (Hackett and Thimann, 1952). All of these observations may 
well be of importance in determining the fundamental action of iodoacetate 
on over-all growth, but at the present time it is impossible to localize the 
basic lesion or process affected.

Inhibition of Fertilization, Egg Cleavage, and Embryogenesis

Inasmuch as much of the work has been done with marine animals, it is 
important to realize that the intracellular concentration of iodoacetate may 
be much less than in the surrounding sea water, and thus that high concen-
trations are often necessary. If the cells are permeable only to the un-ionized 
iodoacetic acid, and if the cells are sufficiently buffered against pH change 
(which is probably true under the conditions), it can be calculated that the 
intracellular concentration will be about 1/400 the external (assuming the 
PH of sea water as 8.2 and of the cell as 6.8). Of course cells may be per-
meable to varying degrees to the ionized iodoacetate.

The effects of iodoacetate on egg and embryo metabolism were discussed 
in earlier sections of this chapter, but inspection of the tables shows that 
relatively little has been done and that the critical enzymes have seldom 
been tested. The resistance of marine egg glycolysis and respiration to iodo-
acetate is sometimes surprising, since it occurs even when the pH is low-
ered, or in extracts or homogenates. One can conclude only that the
3-PGDH of marine eggs is much less sensitive than the mammalian enzyme to iodoacetate. Concentrations above 1 mM, and often 10 mM or above, are required for marked inhibition of respiration and glycolysis.

It is quite clear that fertilization of marine invertebrate eggs is not affected by iodoacetate, even at concentrations of 20-30 mM or when the pH of the sea water is reduced to 6, as was shown for *Urechis caupo* (Tyler and Schultz, 1932), *Arbacia punctulata* (Runnström, 1935), and *Echinus esculentus* (Kriszat and Runnström, 1952); it was concluded that glycolysis is not necessary for fertilization. Indeed, iodoacetate and iodoacetamide at 2 mM promote elevation of the fertilization membrane, due, it was thought, to reaction with SH groups on the plasma membrane with disruption of hydrogen bonds (Isaka and Aikawa, 1963). Early cleavage is also relatively resistant to iodoacetate. When *Urechis* eggs are fertilized and then placed in iodoacetate-containing sea water, there is no effect on the cleavage to the 8-cell stage (Ellis, 1933). However, if the pH of the sea water is lowered to 6, some inhibition is observed: cleavage is depressed 50% and 90% by 1.7 mM and 3.4 mM iodoacetate, respectively, in *Arbacia* eggs (Clowes and Krahl, 1940) and 30 mM causes separation of the cells, due to some action on their surface membranes (Runnström, 1935). We see again a stimulation of division at low concentrations (0.2-0.4 mM) of iodoacetate (Krahl and Clowes, 1940). The egg of the marine snail *Ilyanassa obsoleta* does not cleave readily in 5.4 mM iodoacetate at pH 6.4, the protoplasmic viscosity is doubled, and the egg surface loses its smoothness and becomes pointed and wrinkled (Butros, 1956). Iodoacetamide may penetrate much better than iodoacetate, since 0.1 mM inhibits cleavage of *Echinus* eggs and causes structural changes in the cortex (Kriszat and Runnström, 1952). There is also a decreased protoplasmic viscosity, as determined by centrifugal displacement of mitochondria, and a shortening of the spindle. The most sensitive period is immediately after fertilization, and this was said to correspond to a peak in the glutathione level. Ellis (1933), however, found that whereas iodoacetate at 10 mM reduces the glutathione content of *Urechis* eggs 60%, there is no significant effect on early cleavage, so that he doubted the SH theories of cleavage. Of course glutathione levels are not very reliable indicators of other SH-containing cell components. Brachet (1938) reported that 17-33 mM iodoacetate does not alter early cleavage or development, but arrests frog development at the gastrula stage; we have observed similar behavior in the development of *Strongylocentrotus purpuratus*.

Later developmental abnormalities induced by iodoacetate have not been extensively studied. Runnström (1935) exposed *Arbacia* eggs to 30 mM iodoacetate for 2-6 hr, and they were then fertilized and returned to normal sea water. These eggs developed into larvae with morphological defects: poor development of stomodeum and oral arms is the commonest abnor-
mality in the plutei, and this can be mainly counteracted by pyruvate. The bilateral determination during the early development of Dendraster excentricus is altered by iodoacetate at concentrations around 0.05–1 mM when applied in a gradient (Pease, 1941). Chick embryos explanted to Ringer-glucose medium undergo morphogenesis and differentiation up to the formation of the primitive nervous system and the heart. Iodoacetate at 0.1 mM causes almost immediate cessation of development and complete degeneration, but at 0.02–0.05 mM the effects are more selective, the head fold being the the most sensitive region, as in glucose deficiency (Spratt, 1950). Eventually the entire neuraxis undergoes degeneration, while the heart remains unaffected and continues to beat. Pyruvate counteracts these effects very effectively, indicating that the block may be on the EM pathway. As might be expected, those regions undergoing the greatest differentiation are in general the most sensitive. In connection with the processes of differentiation, the effects of iodoacetate on posterior regeneration in the annelid Tubifex tubifex are interesting, and in addition provide an instance of extreme sensitivity (Anderson, 1956). Although regeneration aerobically is not disturbed by concentrations up to 0.01 mM (indeed some acceleration of later regeneration may be observed), anaerobically a concentration of 0.00001 mM kills all the worms.

**EFFECTS ON NEOPLASTIC GROWTH**

Tumors as a whole have a characteristic metabolic pattern, although deviations from this are well known, and for this reason their response to iodoacetate is interesting. This characteristic pattern can be briefly summarized as follows: (a) a high glycolytic rate, both aerobic and anaerobic (Table 1-36), (b) a normal respiratory rate, despite deficiencies in mitochondrial enzymes such as succinate oxidase, pyruvate oxidase, α-ketoglutarate oxidase, and cytochrome oxidase, (c) a rather low respiratory quotient, (d) a high hexokinase activity (perhaps due to deficiency of the normal control) and an efficient mechanism for the utilization of glycolytically generated ATP for glucose phosphorylation, (e) a frequent exhibition of the Crabtree effect, (f) low levels of glucose-6-phosphatase and phosphoglucomutase with high levels of phosphohexoisomerase, all of which directs the sequence along the EM pathway and away from glycogen formation and other pathways, (g) perhaps a deficiency in phosphorylase, (h) a slightly more active pentose-P pathway than in normal tissues, (i) somewhat low levels of NAD and NADP (which may alter susceptibility of some enzymes to iodoacetate), and (j) a high incorporation rate of amino acids into protein. Many of these characteristics, such as the high glycolytic rates, are not necessarily seen in all proliferating tissues. The responses of certain systems to iodoacetate have been given for tumors in previous tables:
anaerobic glycolysis (Table 1-11), aerobic glycolysis (Table 1-16), glucose utilization (Table 1-22), levels of ATP and creatine-P (Table 1-15), active transport processes (Table 1-31), and others, in which comparisons with normal tissues may be made. The relatively high glycolytic activity raises the possibility that tumor tissue is particularly sensitive to iodoacetate, as is the retina, but by itself does not make this necessarily so.

Comparison of Metabolic Responses in Tumors and Normal Tissues

A few investigators have compared inhibitions in tumors and nontumor tissues and, although the interpretation of the results is difficult, it may be of interest to summarize this work. Glycolysis and glucose utilization in tumors are generally depressed more than in normal tissues: Jensen rat sarcoma and rat testis (Krebs, 1931), mouse sarcoma and muscle (Gerard, 1931), rat sarcoma and chick embryo connective tissue (Krontowski et al., 1932 a, b), mouse Sarcoma 180 and muscle (Scharles et al., 1935), and Gardner mouse lymphosarcoma and lymphatic cells (Villavicencio and Barron, 1957). For example, glucose utilization by rat sarcoma is inhibited 70% by 0.036 mM bromoacetate, whereas only 5% inhibition is seen in fibroblasts. Glycolysis in extracts of mouse sarcoma is inhibited 61% by 1 mM iodoacetate with fructose-1,6-diP as the substrate, whereas only 16% inhibition is seen in comparable muscle extracts. Aerobic glycolysis in mouse lymphosarcoma is inhibited 85% by 0.01 mM iodoacetate, but only 63% in lymphatic cells. Similar differences have been noted in phosphate uptake and esterification of phosphate (Scharles et al., 1935; Clowes and Keltch, 1952). In some instances the relative sensitivity may depend on the iodoacetate concentration (Young and Taylor, 1953). The validity of such comparisons is doubtful, however, inasmuch as one should ideally use tumors and homologous tissues (i.e., the same tissue in a normal and in a neoplastic state). Another problem arises when extracts are used, since the metabolic patterns of glucose utilization are quite different in extracts of tumors and normal tissues, due mainly to the discrepancy between the ATPase activities. Certainly inspection of the tables previously presented does not give the impression that tumor tissue is particularly sensitive to iodoacetate, nor is there adequate evidence by which to compare the relative susceptibilities of 3-PGDH from tumors and normal tissues.

There is no doubt that the EM pathway is readily blocked in a variety of tumors by low concentrations of iodoacetate. The following may be mentioned, in addition to those given above: mouse carcinoma (Harrison and Mellonby, 1931), Jensen rat sarcoma (Crabtree and Cramer, 1933 a), Kato spindle cell sarcoma (Tsuzuki, 1936), Yoshida sarcoma (Holzer et al., 1958; Schmidt, 1961), Ehrlich ascites carcinoma (Holzer, 1956; Laws and Stickland, 1962), and human mammary fibrosarcoma (van Vals et al., 1956). It may be worthwhile to mention briefly some of the metabolic responses
to iodoacetate in tumors, especially those more characteristic of neoplastic tissue and possibly relating to the inhibition of growth. Glucose often depresses respiration in tumors (Crabtree effect) and this is apparently due to compartmentalization of ATP formed from glycolysis, and the replacement of the ATP lost in the inhibited respiration by ATP generated glycolytically. The basis for this may be some competition between reactions for ADP or P; This phenomenon is seen well in Ehrlich ascites carcinoma cells. If iodoacetate prevents ATP generation during glycolysis, one might expect it to stimulate respiration in such cells at low concentrations and in the presence of glucose. The results reported are not consistent. Ibsen et al. (1958) stated that iodoacetate does not stimulate the respiration under these circumstances; slight stimulation was observed in a few cases, but it is questionable if the change is significant. Kvamme (1958 b) found only a 17% inhibition of respiration by 0.05 mM iodoacetate. Laws and Stickland (1962), however, obtained a 14% increase in the respiration by 1 mM iodoacetate. To add further complication, Seelich and Letnansky (1960) reported definite respiratory stimulation by 0.5 mM iodoacetate in phosphate buffer, but only an inhibition in bicarbonate buffer. The interrelationships in this field are so complex that it is perhaps not surprising that variable results are often obtained. Another factor which must be considered in interpreting the Crabtree effect and the action of iodoacetate is the role that NADP reduction and the pentose-P pathway play in determining the respiratory rate. In any event, it is clear that iodoacetate markedly reduces the levels of ATP and total adenine nucleotides in ascites cells in the presence of glucose (Kvamme, 1958 a; Thomson et al., 1960). The marked fall in the pH, brought about in tumor cell suspensions by the addition of glucose, is not only abolished by low concentrations of iodoacetate, but a rise in the pH is observed, e.g., from 6.95 to 7.48 (Racker, 1956) or 5.92 to 7.07 (Kvamme, 1958 b), the magnitude depending on the glucose concentration and the incubation time. The effects of such pH changes intracellularly on neoplastic growth are not known. The ratio of C\textsubscript{14}\textsubscript{O\textsubscript{2}} formed from glucose-1-C\textsubscript{14} to that from glucose-6-C\textsubscript{14} is markedly reduced in ascites cells (Table 1-20) (Wenner, 1959) and, as was described (page 131), this is due here to an indirect depression of the pentose-P pathway through the block of pyruvate formation, since the principal oxidizer of NADPH in these cells is pyruvate. Finally, it may be recalled that 0.54 mM iodoacetate inhibits the incorporation of acetate-1-C\textsubscript{14} into proteins, fatty acids, and cholesterol in slices of a variety of tumors (van Vals and Emmelot, 1957), although there is marked variation between the different tumors, e.g., incorporation into fatty acids being inhibited 85% in adrenal carcinoma and not at all in hepatoma. Such interferences in biosynthesis, whatever the mechanisms involved, must be important in the effects of iodoacetate on neoplastic growth. The inhibition of inward transport and accumulation of amino
acids in lymphosarcoma (Kit and Greenberg, 1951) and ascites carcinoma cells (Christensen and Riggs, 1952; Tenenhouse and Quastel, 1960) also may constitute a mechanism for growth retardation.

**Neoplastic Growth in Vitro**

When sarcoma fragments are incubated with 0.018 mM bromoacetate for 1 hr and then placed in tissue culture, growth is inhibited by 38%; at 0.036 mM the inhibition is 63%, and at 0.072 mM there is no growth (Krontowski et al., 1932 b). Iodoacetate is more depressant and complete growth inhibition is seen with 0.01 mM. Transplantability is not impaired until much higher concentrations are reached. It was emphasized that the sarcoma is more sensitive to these inhibitors than are cultures of fibroblasts. Cultures of Earle’s strain L cells are also completely inhibited by 0.01 mM iodoacetate (Cailleau et al., 1955). Eagle’s KB strain of human carcinoma cells seems to be the most sensitive of all, 50% growth reduction being produced by 0.0022 mM iodoacetate (Smith et al., 1959). The Ehrlich ascites carcinoma is likewise quite sensitive, inasmuch as incubation with 0.05 mM iodoacetate followed by inoculation into mice leads to some depression of subsequent growth (Holzer et al., 1955 c; Holzer, 1956). Parallel inhibitions of glycolysis and growth were observed, leading Holzer to support the concept of the necessity of glycolysis for tumor growth. All of these results point to the high sensitivity of neoplastic cells to iodoacetate and at least present the possibility of selective depression of growth in the animal.

**Neoplastic Growth in Vivo**

Harrison and Mellanby (1931) showed that injection of toxic doses of iodoacetate into mice bearing Carcinoma 63 leads to a marked inhibition of glycolysis in the tumor, although subtoxic doses apparently have no effect. Administration of bromoacetate and iodoacetate to mice and rats bearing various tumors leads to interesting but inconsistent results (Krontowski et al., 1933). Implanted adenocarcinoma responds slowly and its growth may be either stimulated or depressed according to the strain used, whereas rat sarcoma is stimulated, and Ehrlich mouse carcinoma is somewhat inhibited by doses which are toxic. Some regression of Flexner-Jobling rat carcinoma and mouse Carcinoma 63 can be observed when iodoacetate is injected subcutaneously or intraperitoneally at doses of 14.5 mg/kg in rats and 29 mg/kg in mice 3 times a week (Goldfeder, 1933). When ammonium chloride is given simultaneously to acidify the animals, the results are more favorable: in 45 mice 8 tumors showed regression and 9 disappeared, and in 29 rats 6 regressed and 9 disappeared. Differences between tumors were also remarked by Franks et al. (1934), mouse Sarcoma 180 showing some regression but Carcinoma 63 being unaffected by injection of iodoacetate into
the tumors. Selle and Bodansky (1935) reasoned that bromoacetate might not penetrate into the tumors and hence tried bromocaproate injected in olive oil subcutaneously, but found no inhibition of rat Sarcoma 39, which is not surprising since bromocaproate is, as far as one knows, not a good glycolytic inhibitor.

Iodoacetate injected intraperitoneally into rats bearing Tumor 256 at a dose of 7 mg/kg/day causes some reduction in over-all growth rate of the animals and also some retardation of tumor growth, but apparently little selectivity is evident (Brunschwig et al., 1946). Iodoacetamide administered in the same manner to mice bearing Sarcoma 37 causes some regression or retardation of tumor growth, this being particularly significant when the tumors are initially large, untreated tumors weighing 3.7 times as much as the tumors in treated animals (Friedman and Rutenberg, 1950 b). Summarizing all the results, one might conclude that a degree of selectivity may be attained with certain types of tumor, although appreciable regression of the tumors is usually seen only when some toxicity also occurs. Although these limited observations with iodoacetate are not especially encouraging, quite possibly specific tumors might respond well enough so that the use of iodoacetate, at least in conjunction with other carcinostatic agents might be entertained. One must remember that low doses of iodoacetate may possibly stimulate tumor growth, since this seems to be a characteristic of this type of inhibitor, so that intensive therapy is indicated.

The only clinical test of iodoacetate was reasonably encouraging (Black and Kleiner, 1947; Black et al., 1947). Given to patients at a dosage of 60–90 mg/day orally (which one may note is around 1 mg/kg/day and a dosage much lower than in experimental animals), it exert suppressive effects on lymphosarcoma, myeloblastic leukemia, carcinoma liver metastases, and other neoplasms. In a number of cases there is hematological remission for several months, or a shrinkage in tumor mass with relief from pain and general weight gain. However, as is the case with most carcinostatic agents, tolerance develops and the tumor escapes. Nevertheless, these results seem to justify further work, especially using iodoacetate in combination therapy rather than sequentially.

Various interesting bifunctional derivatives of iodoacetate and iodoacetamide were synthesized and tested by Kramer et al. (1963), on the basis that they are highly active alkylating agents and might be cross-linking. A typical compound is ethylenebis(iodoacetate):

\[ \text{I—CH}_2—\text{CO—O—CH}_2\text{CH}_2—\text{O—CO—CH}_2—\text{I}, \]

which hydrolyzes slowly in physiological media (half-life of 80 min in phosphate buffer at pH 7.4 and 37°C) but more rapidly in serum or tissue extracts. The enzymic and metabolic effects of such compounds are unknown, but the compound above (known as S-10) is 4–8 times as toxic as iodoacet-
Effects on Neoplastic Growth

Attempts have been made to increase or decrease the susceptibility of cells to radiations, depending on whether one wishes to kill or protect the cells. Anaerobiosis decreases and cyanide increases the susceptibility of tumor cells to X-radiation, but Crabtree and Cramer (1933 b) could observe no effects of either iodoacetate or fluoride. Franks et al. (1934) postulated that radiation damages oxidative systems in cells and therefore that radiated cells may be more dependent on glycolysis, which led them to determine regressions in two types of tumor (see accompanying tabulation). It may be that exposure to radiation increases the sensitivity of tumor C-180 to iodoacetate, but the effect is not marked. The aerobic glycolysis of tumor C-180 is greater than that of the other tumor, which may account for the effect. If E. coli cells are irradiated with ultraviolet light and then treated with 0.2 mM iodoacetate for 15 min, the degree of survival is greater than without iodoacetate (Wainwright and Mullaney, 1953). The mean increase in viable cells produced by iodoacetate is around 128%. On the other hand, the mortality of mice is increased by a combination of iodoacetate and X-radiation (see accompanying tabulation) (Feinstein et al., 1954).

<table>
<thead>
<tr>
<th>X-radiation</th>
<th>Iodoacetate</th>
<th>% Regressions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Tumor C-63</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tumor C-180</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>2.7</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>2.0</td>
</tr>
</tbody>
</table>

The dose of iodoacetate was 20 mg/kg. Quintiliani et al. (1961) found that iodoacetate can sensitize mice and rats to ionizing radiations when given intravenously at 40 mg/kg. The iodoacetate was also found to inhibit the
uptake of labeled phosphate into the DNA of radiosensitive tissues and regenerating liver, so that the effects of radiation and iodoacetate are additive. It is often difficult to interpret the results of such work as presented in this section, inasmuch as ordinary and expected summation of effects is not distinguished from true potentiation.

**EFFECTS ON THE GROWTH OF MICROORGANISMS**

The value of simple bacteriostatic studies with iodoacetate is only that bacteria differ so much in inherent metabolic patterns that it is interesting to compare their relative sensitivities on this basis. In addition, there is the possibility of using iodoacetate or derivatives as antiseptics. One must be particularly careful in attributing growth inhibition in microorganisms to a block of the EM pathway, because there may be other susceptible pathways, and furthermore actions on the cell surface or transport systems are quite possible. Bacteria are also very often grown in complex culture media providing substrates which may be utilized to circumvent a block of the EM pathway.

**Bacteria**

Some idea of the general susceptibility of bacteria to iodoacetate may be obtained by giving a few selected results expressing inhibitory concentrations: *Staphylococcus aureus* is inhibited 50% by 0.063 mM (Yanagita, 1947), various homofermentative lactobacilli are minimally inhibited by 1 mM and heterofermentative lactobacilli by 2.5 mM (Fitzgerald and Jordan, 1953), *Enterococcus* is inhibited minimally by 0.05 mM and markedly by 0.1 mM (Meyer, 1932), *Proteus vulgaris* is inhibited somewhat by 2 mM (Kandler et al., 1956), *Escherichia coli* is inhibited 50% by 0.11 mM (Loveless et al., 1954), *Thiobacillus thiooxidans* is completely inhibited by 0.01 mM (Vogler et al., 1942), and *Mycoplasma gallisepticum* is not inhibited by 0.1 mM, although the stimulation of growth by glucose is inhibited (Gill, 1962). It is interesting that the high sensitivity of *T. thiooxidans* is associated with a very marked inhibition of sulfur oxidation by iodoacetate. Various derivatives of bromoacetate, for example the benzyl ester, have been used as antiseptics commercially, as in wine making, under the trade names of Antiferm and Fermacid (Eeckhout, 1948; Loncin, 1950; Dal-Cin, 1950). Germination of *Bacillus coagulans* spores induced by alanine is resistant to 5 mM iodoacetate, but germination brought about by glucose in heated spores is 98% inhibited, so that the activation in the latter case must be through glycolysis (Amaha and Nakahara, 1959). Flagellar motility in *Bacillus brevis* is inhibited rapidly by iodoacetate and iodoacetamide, but high concentrations (10 mM) were used (De Robertis and Pelullo, 1951).
Little is known of the responses of bacterial infections to iodoacetate. Administration of iodoacetate prolongs the life of guinea pigs given subcutaneous inoculations of Bacillus anthracis, and this was attributed to an inhibition of certain proteinases involved in the virulence of these bacteria (Wohlfteil and Wollenberg, 1938). It has been stated that iodoacetate given to tuberculous guinea pigs has no effect on the bacteria but inhibits the breakdown of the infected tissue, thus reducing the spread of the infection (Mauer and Kinkeldy, 1953). Combination with isoniazid suppresses dissemination of the infection better than with either substance alone. Investigations such as those by Berry and co-workers on infections, using malonate, would perhaps provide interesting results on the complex mechanisms involved in bacterial growth, invasiveness, and host responses.

**Viruses and Bacteriophages**

It is evident that effects mediated through EM pathway inhibition must be on the host cells since viruses do not possess the glycolytic enzymes, and that depression of viral proliferation by this mechanism must be the result of a decrease in the generation of ATP and consequent interference with syntheses required for the formation of virus. On the other hand, some viruses may have reactive SH groups, alkylation of which would so modify virus structure as to impede duplication. Incubation of tobacco mosaic virus with high concentrations of iodoacetamide (50–100 mM) at pH 8 leads to partial inactivation of the virus, but under these conditions it is impossible to say what groups are reacted (Anson and Stanley, 1941). The virus is obviously quite resistant. Influenza virus PR-8 is also resistant since 50 mM iodoacetamide at pH 7 has no effect before 8 days (Knight and Stanley, 1944). No direct effects on mouse pneumonia virus by 10 mM iodoacetamide were observed (Volkert and Horsfall, 1947). Larger more complex viruses seem to be much more susceptible. Psittacosis virus is partially inactivated by 2.5 mM iodoacetamide, but not at all by 4.5 mM iodoacetate, incubation being 1 hr at 37° (Burney and Golub, 1948). The inactivation of vaccinia and fowl plague viruses by SH reagents is first order at 0.1 mM; iodoacetamide reduces the virus titer markedly (Allison, 1962). The primary attachment of the virus to the host cell and its penetration are not affected, and possibly the uncoating of the virus preceding multiplication is prevented. Allison et al. (1962) made an extensive study of the susceptibility of many viruses to iodoacetamide, the viruses being exposed to the inhibitor for 1 hr at 18° and pH 7.4. Certain viruses, such as vaccinia, influenza A-MEL, and Newcastle, appear to be quite sensitive to iodoacetamide since 0.1 mM reduces their titer several hundredfold. On the other hand, Coxsackie and Echo 7 viruses are completely resistant to 1 mM iodoacetamide. Thus there is evidence that iodoacetamide can directly modify viruses to reduce their infectivity or proliferative activity, but nothing is
yet known of the groups involved or the nature of the primary interference in the complex sequence of virus multiplication.

Some work has been done on the application of these inhibitors to host-virus preparations. The number of lesions in detached leaves produced by tobacco mosaic virus is reduced slightly by 0.05 mM iodoacetate and quite markedly by 0.3 mM (Chiba et al., 1953). Since azide and 2,4-dinitrophenol do not reduce the lesion density, one suspects that mere reduction in ATP cannot explain the effect of iodoacetate. Iodoacetate does not inhibit in the dark, and may increase the number of lesions, so that an effect on photosynthesis is a possibility. The proliferation of vaccinia virus in chick embryonic tissue over 4 days is markedly depressed by 0.054 mM iodoacetate (R.L. Thompson, 1947). The yield of foot-and-mouth disease virus from cultures of kidney cells is reduced 10% by 0.01 mM and 33% by 0.1 mM iodoacetate, although there is no delay in the appearance of the virus (Potlatnick and Bachrach, 1960). The effects are not counteracted by pyruvate, so the site of attack may not be the EM pathway, although it could also mean that virus multiplication is in some way dependent directly on glycolysis.

Turning now to bacteriophages, we shall examine first the results which have been obtained on coliphages. Spizizen (1943) allowed the phage to adsorb onto the bacterial cells, washed the cells, and then placed them in nutrient media which allowed phage production. Iodoacetate at 0.5 mM completely abolished phage multiplication whatever the medium used. Although inhibition of phage multiplication has been generally observed, it seems clear that iodoacetate is not selective and that bacterial growth is simultaneously depressed (Ryzhkov and Semich, 1947; Czekalowski, 1952; Dolby, 1955). Low concentrations of iodoacetate (0.05 mM) block phage proliferation and reduce bacterial growth, whereas higher concentrations (0.25–1.35 mM) destroy both phage and bacteria. There may be a greater effect on phage, however, as indicated in the study of Dolby, in which the inhibition index for T2 coliphage is 0.03 at 0.05 mM iodoacetate and for the bacteria is 0.77, this pointing to a differential, if not a selective, effect. Baer et al. (1955) found that the addition of 1 mM iodoacetate to three host-virus systems, including coliphage, at various times during the proliferation of phage leads to immediate and complete inhibition of further phage formation, so that the final phage yields correspond to the amount present when the inhibitor is added; they suggested that iodoacetate could be used to titrate mature intracellular phage at any time. There is no effect on free phage or on its attachment or penetration into the bacteria.

The development of other phages is well inhibited by iodoacetate. If staphylococci containing “phage precursor” are treated with 0.1 mM iodoacetate for over 15 min, there is a marked reduction of the phage yield (Krueger and Scribner, 1940). Phage precursor can be inactivated without
killing the bacteria, but no concentration of iodoacetate which inhibits phage proliferation and does not depress bacterial growth could be found. Price (1947) reported that 1 mM iodoacetate reduced staphylococcal ATP and phage multiplication and, taken with the results of other inhibitors, suggested that the mechanism is simply a depletion of energy for phage synthesis. Now that the nature of phage proliferation is fairly well understood, there is need of further investigation of the actions of iodoacetate and related inhibitors in order to localize the site of action and to characterize more accurately the role of the host cell metabolism in the formation of phage.

Yeasts and Fungi

Rapkine (1937) reported that yeast growth is essentially completely inhibited by iodoacetate at concentrations around 0.3 mM and at pH 4.5, which is not surprising since fermentation is abolished. The concentration for 50% inhibition of growth was put at 0.011 mM by Loveless et al. (1954), yeast being 10 times as sensitive as E. coli. The growth inhibition is partially overcome by acetate and glutamate, but not by pyruvate (Schmid, 1958). Actually, the effect of the pH is so marked that it is useless to compare the results of different workers. Aldous (1948) demonstrated clearly that 1 mM iodoacetate inhibits completely at pH 3.9 or below, 50% around pH 4.6, and not at all above a pH of 5. How much of the inhibition is due to nonspecific damage by penetrating acid is not known. Hansen (1956 a) showed the same relationship for bromoacetate, the concentration for 100% growth inhibition of Torula utilis being 4.32 mM at pH 5.2, and 0.079 mM at pH 3.2.

Halogenated fatty acids have been used commercially for their fungistatic activity and again it is found that the effectiveness depends on the pH (Hoffman et al., 1940). Shirk and Gertler (1958) investigated the relative susceptibilities of several fungi to bromoacetate, and found 50% growth inhibition to be given by concentrations between 0.1 and 0.5 mM, with no marked differences between the organisms (Aspergillus, Penicillium, Pullularia, Myrothecium, and Trichoderma). Aspergillus niger growth is arrested by iodoacetate and this is reversed by D-3-P-glycerate but not by cycle intermediates (Behal, 1959). This fungus can convert 3-P-glycerate to hydroxybutyrate, which can also counteract iodoacetate inhibition, indicating that in this organism there are pathways diverging from the EM pathway which are important for growth (Behal, 1960). Wheat leaf rust (Puccinia recondita) development is inhibited by concentrations of iodoacetate above 0.5 mM, but there is also progressive damage to the leaf, so that a selective action on the mold is not possible. If it is possible at all to make comparisons, it seems that fungi are somewhat less sensitive than most bacteria to iodoacetate, but this could be related to a better penetration of the inhibitor into the bacteria.
Protozoa

The results reported on ciliates are quite variable. Wertheimer (1932) found the motility of Paramecium caudatum to be uninfluenced by bromoacetate, even in the presence of cyanide, but Calcutt (1950) claimed that 0.5 mM iodoacetate kills 90% of the cells of Paramecium bursaria within 32 min in the dark and 14 min in the light. The effect of light was believed to be due to its making more SH groups available for reaction. The pH must be important here but in neither work was it given. The motility of Tetrahymena pyriformis is unaffected by 0.03 mM iodoacetate, reduced by 0.1 mM, and abolished by 0.3 mM within 60 min, this being apparently at pH 7.3 (Ryley, 1952). It would be interesting to know if there is direct action on the cilia and their contractile elements, but in none of the work can correlations be made.

Trypanosomes are apparently very sensitive to iodoacetate, so much so that iodoacetate can be used as a chemotherapeutic agent. Smythe and Reiner (1933) first showed that 1 mM iodoacetate kills Trypanosoma equiperdum within 2–3 min. Injection of tolerated doses of iodoacetate into infected rats leads to a clearing of the blood of the organisms in 2–3 hr; the blood remains free of the trypanosomes for several days, and then they reappear. Further doses clear the blood repeatedly but do not eradicate the organisms. Von Jancsó and Von Jancsó (1936) confirmed these observations for both iodoacetate and bromoacetate, and noted a progressive slowing of motility until the organisms disappear from the blood. T. lewisi motility is abolished by 0.1 mM iodoacetate at a pH of 7.3, respiration being simultaneously depressed 50% (Ryley, 1951). T. vivax is apparently sensitive to the same degree (Desowitz, 1956). T. rhodesiense is killed by 0.03 mM iodoacetate (Williamson, 1959 b). The facts that trypanosomes are inhibited so readily at higher pH’s and that iodoacetamide is no more potent than iodoacetate indicate that penetration of the inhibitor is not seriously impeded. Entamoeba histolytica does not survive in 0.04 mM iodoacetate but the associated bacteria are inhibited only some 32% (Yang, 1959). The addition of pyruvate counteracts the depression of the bacterial growth but does not alter the inhibition of the amebae, and because of this it was concluded that a direct action of the iodoacetate on the amebae is likely. Certainly it does not seem to be through an inhibition of the bacteria.

COMPARISON OF HALOGENATED ACIDS AND THEIR DERIVATIVES

It is quite possible that for certain purposes iodoacetate is not the ideal alkylating agent, and thus in this section we shall compare iodoacetate with iodoacetamide, with bromoacetate and chloroacetate, and with various
esters. In addition, some of the other halogenated alkylating agents will be mentioned.

**Iodoacetate and Iodoacetamide**

Goddard (1935) introduced iodoacetamide as a more penetrable alkylating agent of the iodoacetate type, and attributed the differences he observed in the responses to these two inhibitors of *Neurospora* ascospores to a greater penetration of the iodoacetamide, although this evidence is inconclusive since the potencies of action within the cells might be different. The important differences in general reactivity may be summarized as follows: iodoacetamide reacts more rapidly than iodoacetate with SH groups (around 1.9 times as fast at pH 7.1 and 4 times as fast at pH 6.1) (Smythe; 1936), whereas iodoacetate usually reacts more rapidly than iodoacetamide with amino groups (Table 1-2). Following reaction with proteins or enzymes, iodoacetate introduces negatively charged groups (—CH₂COO⁻), while iodoacetamide introduces neutral groups (—CH₂CONH₂), and this might be quite important in the effects produced. If enzyme SH groups are involved in the inhibition, one might expect iodoacetamide to inhibit more rapidly and perhaps more potently than iodoacetate, and this is usually observed (Table 1-41). As far as I know, no direct comparison of the relative actions on 3-PGDH has yet been reported, which is unfortunate since it might help to understand the differences in the inhibition of the EM pathway.

When cellular systems are considered there is in addition to these factors the matter of relative penetrations into the cells and the resultant intracellular concentration. The potency of iodoacetate should vary markedly with the pH, except as membrane structure is altered (the pKₐ of iodoacetamide is far too low for protonation in any usable pH range). Theoretically one would expect iodoacetamide to be much more potent than iodoacetate at pH 7 or above and, from the results on isolated enzymes, would not predict iodoacetate ever to be much more effective than iodoacetamide even at low pH's. However, these predictions are not borne out, and in several cases iodoacetate is the more potent, even around neutrality (Table 1-42). Indeed, with respect to the inhibition of glycolysis and respiration, iodoacetate appears to be generally more effective. The inhibition of photosynthesis, on the other hand, follows the theory quite closely, although at low pH's iodoacetate is often a good deal more potent than iodoacetamide, which may be due to the fact that when the external pH is much below the intracellular pH, the concentration of iodoacetate inside the cells can be greater than outside (see page 1-714). Stannard (1937) long ago suggested that the actions of iodoacetate and iodoacetamide are in some way different, particularly because they have quite different effects on the R.Q. of muscle. Furthermore, iodoacetate causes an increase in the muscle excitability, followed by a fall during contracture, whereas iodoacetamide does not alter the excitability until rigor occurs (Stannard, 1938 b). Lehninger (1951) found
### Table 1-41
### Comparison of Iodoacetate and Iodoacetamide on Enzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Iodoacetate (mM)</th>
<th>% Inhibition</th>
<th>Iodoacetamide (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol dehydrogenase</td>
<td>Yeast</td>
<td>1</td>
<td>30</td>
<td>1</td>
<td>41</td>
<td>Barron and Levine (1952)</td>
</tr>
<tr>
<td>Amylase</td>
<td>Human saliva</td>
<td>1</td>
<td>25</td>
<td>1</td>
<td>30</td>
<td>Schneyer (1952)</td>
</tr>
<tr>
<td>Betaine aldehyde dehydrogenase</td>
<td>Rat liver</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>40</td>
<td>Rothschild and Barron (1954)</td>
</tr>
<tr>
<td>Choline oxidase</td>
<td>Rat liver</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>40</td>
<td>Rothschild et al. (1954)</td>
</tr>
<tr>
<td>Glycolate oxidase</td>
<td>Barley</td>
<td>10</td>
<td>46</td>
<td>10</td>
<td>20</td>
<td>Noll and Burris (1954)</td>
</tr>
<tr>
<td>Hydrogenlyase</td>
<td><em>Aerobacillus polymyza</em></td>
<td>2</td>
<td>70</td>
<td>2</td>
<td>89</td>
<td>Crewther (1953)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>95</td>
<td>40</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>a-Ketoglutarate oxidase</td>
<td>Mosquito particles</td>
<td>0.1</td>
<td>55</td>
<td>0.1</td>
<td>54</td>
<td>Gonda et al. (1957)</td>
</tr>
<tr>
<td>Malonyl-CoA-Co₂ exchange enzyme</td>
<td><em>Clostridium kluyveri</em></td>
<td>0.1</td>
<td>25</td>
<td>0.1</td>
<td>100</td>
<td>Vagelos and Alberts (1960)</td>
</tr>
<tr>
<td>NADH oxidase</td>
<td>Mosquito particles</td>
<td>0.1</td>
<td>10</td>
<td>0.1</td>
<td>25</td>
<td>Gonda et al. (1957)</td>
</tr>
<tr>
<td>NADH:ferri cyanide oxidoreductase</td>
<td>Pig liver</td>
<td>1</td>
<td>21</td>
<td>1</td>
<td>87</td>
<td>Mahler et al. (1958)</td>
</tr>
<tr>
<td>Proteinase</td>
<td><em>Clostridium histolyticum</em></td>
<td>6.7</td>
<td>19</td>
<td>6.7</td>
<td>30</td>
<td>Kocholaty and Krejci (1948)</td>
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<tr>
<td>Pyruvate decarboxylase</td>
<td>Yeast</td>
<td>5</td>
<td>60</td>
<td>5</td>
<td>73</td>
<td>Barron and Singer (1945)</td>
</tr>
<tr>
<td>Pyruvate oxidase</td>
<td>Pigeon brain</td>
<td>0.25</td>
<td>50</td>
<td>0.05</td>
<td>50</td>
<td>Peters and Wakelin (1949)</td>
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<td>Ribonuclease</td>
<td>Beef pancreas</td>
<td>0.3</td>
<td>36</td>
<td>0.3</td>
<td>13</td>
<td>Zittle (1946)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.6</td>
<td>66</td>
<td>0.6</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Serine deaminase</td>
<td><em>Escherichia coli</em></td>
<td>0.054</td>
<td>0</td>
<td>0.054</td>
<td>38</td>
<td>Lenti and Grillo (1955)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.54</td>
<td>34</td>
<td>0.54</td>
<td>65</td>
<td></td>
</tr>
<tr>
<td>Δ¹-Steroid dehydrogenase</td>
<td><em>Pseudomonas testosteroni</em></td>
<td>1</td>
<td>17</td>
<td>1</td>
<td>17</td>
<td>Levy and Talalay (1959)</td>
</tr>
<tr>
<td>Δ⁴,5α-Steroid dehydrogenase</td>
<td><em>Pseudomonas testosteroni</em></td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>11</td>
<td>Levy and Talalay (1959)</td>
</tr>
<tr>
<td>Succinate oxidase</td>
<td>Rat liver</td>
<td>1</td>
<td>52</td>
<td>1</td>
<td>90</td>
<td>Potter and DuBois (1943)</td>
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<td></td>
<td>Pigeon breast muscle</td>
<td>1</td>
<td>42</td>
<td>5</td>
<td>64</td>
<td>Barron and Singer (1945)</td>
</tr>
<tr>
<td>Tripeptidase</td>
<td>Human erythrocytes</td>
<td>10</td>
<td>40</td>
<td>10</td>
<td>73</td>
<td>Tsuboi et al. (1957)</td>
</tr>
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</table>
### Table 1.42

<table>
<thead>
<tr>
<th>Activity measured</th>
<th>% Iodoacetamide</th>
<th>Iodoacetate (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolysis in frog muscle (at 2 hr)</td>
<td>100</td>
<td>0.1</td>
<td>2.7</td>
<td>Stannard (1932)</td>
</tr>
<tr>
<td>Glycolysis in <em>E. coli</em> (PH 7.4)</td>
<td>88</td>
<td>1</td>
<td>1</td>
<td>Agosin et al. (1957)</td>
</tr>
<tr>
<td>Respiration (endogenous) of <em>E. coli</em> (PH 7.4)</td>
<td>76</td>
<td>5</td>
<td>3</td>
<td>Agosin et al. (1957)</td>
</tr>
<tr>
<td>Respiration (endogenous) of <em>Neurospora</em> asciopores (PH 5.9 at 3-5.5 hr postactivation)</td>
<td>53</td>
<td>5</td>
<td>5</td>
<td>Godiard (1935)</td>
</tr>
<tr>
<td>Fermentation in yeast</td>
<td>100</td>
<td>5</td>
<td>3</td>
<td>Sayle (1936)</td>
</tr>
<tr>
<td>Photosynthesis in <em>Chlorella</em> at:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH 4.6</td>
<td>0.014</td>
<td>2.3</td>
<td>100</td>
<td>Fraser (1954)</td>
</tr>
<tr>
<td>pH 6.5</td>
<td>0.048</td>
<td>50</td>
<td>50</td>
<td>Fraser (1954)</td>
</tr>
<tr>
<td>Photosynthesis in <em>Chlorella</em> (PH 9)</td>
<td>0.027</td>
<td>2.7</td>
<td>100</td>
<td>Fraser (1954)</td>
</tr>
<tr>
<td>Photosynthesis in <em>Chlorella</em> (PH 5.3)</td>
<td>0.014</td>
<td>2.7</td>
<td>100</td>
<td>Fraser (1954)</td>
</tr>
<tr>
<td>Photosynthetic incorporation of <em>C</em>14O2 into tobacco leaves (PH 5.3 at 30 sec)</td>
<td>0.015</td>
<td>2.7</td>
<td>100</td>
<td>Fraser (1954)</td>
</tr>
<tr>
<td>Block of chitotrop effect of epinephrine on frog heart (PH 7)</td>
<td>0.027</td>
<td>1.5</td>
<td>30</td>
<td>Fraser (1954)</td>
</tr>
<tr>
<td>Growth of <em>E. coli</em></td>
<td>0.016</td>
<td>1.5</td>
<td>30</td>
<td>Fraser (1954)</td>
</tr>
<tr>
<td>Psittacosis virus survival (PH 7)</td>
<td>0.011</td>
<td>0.5</td>
<td>30</td>
<td>Fraser (1954)</td>
</tr>
</tbody>
</table>
that iodoacetamide uncouples mitochondrial oxidative phosphorylation, while iodoacetate does not; he postulated some permeability barrier although this does not now seem very likely. The blocking action of iodoacetate on the cardiostimulatory action of epinephrine (Table 1-42) and the absence of an effect of iodoacetamide at 50 times the concentration are also difficult to explain. One must have more comparative studies of the actions on isolated glycolytic enzymes and especially 3-PGDH before some of the unpredicted results can be reasonably interpreted. Some of the differences may arise from the group charge introduced by iodoacetate and the absence of this with iodoacetamide, but it does not seem possible to explain all the data on this basis.

The effects of iodoacetate and iodoacetamide on cardiac mitochondrial oxidations (Table 1-14) and atrial characteristics (Table 1-35) have been discussed. In contrast to most of the results on isolated enzymes, many mitochondrial oxidations are somewhat more effectively inhibited by iodoacetate, but few consistent correlations can be made. The effects on the atria are often quite different, both quantitatively and qualitatively, but certainly iodoacetamide is not markedly more potent, although the pH was 7.4, i.e., there is no evidence that penetration is a factor, which might be interpreted as indicating that much of the action is on the cell membranes.

Iodoacetate and the Other Haloacetates

The important differences between the haloacetates may be summarized as follows: (1) bromoacetic and chloroacetic acids are somewhat stronger acids than iodoacetic acid, and hence would not be expected to penetrate better, and (2) bromoacetate reacts a little slower than iodoacetate with SH groups, whereas chloroacetate reacts very slowly. Fluoroacetate is omitted from discussion here because it is completely unlike iodoacetate. One would thus predict bromoacetate to act much like iodoacetate, although somewhat less potently, and chloroacetate to have little activity as an alkylating agent or glycolytic inhibitor. These predictions are borne out in all the work reported. Bromoacetate exhibits similar toxic effects on animals compared to iodoacetate (Steinauer, 1874; Dalgaard-Mikkelsen et al., 1955), also inhibits many SH enzymes (Table 1-43), inhibits the EM pathway readily, and produces muscle rigor. The effects of chloroacetate on animals are quite different and probably related to other sites of action (Fuhrman et al., 1955). Some selected relative potencies for these three haloacetates are given in Table 1-44. Although bromoacetate is an effective alkylating agent, there seems to be no evidence that it possesses advantages over iodoacetate for this purpose.

Bromo-, chloro-, and iodo-substituted aliphatic acids of longer chain length than acetic acid are relatively weak or completely ineffective alkylating agents, not reacting with SH groups (Schroeder et al., 1933 b) or
<table>
<thead>
<tr>
<th>Enzyme or metabolism</th>
<th>% Inhibition by:</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Concentration (mM)</td>
<td></td>
</tr>
<tr>
<td>Papain (pancreatic)</td>
<td>1</td>
<td>Bersin and Logemann (1933)</td>
</tr>
<tr>
<td>Lipase (pancreatic)</td>
<td>2.7</td>
<td>Weinstein and Wyne (1930)</td>
</tr>
<tr>
<td>Transamination (pigeon muscle)</td>
<td>2</td>
<td>Cohen (1939)</td>
</tr>
<tr>
<td>Respiration (rat brain slices)</td>
<td>0.1</td>
<td>Fuhrman et al. (1955)</td>
</tr>
<tr>
<td>Glycolysis (rat brain slices)</td>
<td>0.1</td>
<td>Fuhrman et al. (1955)</td>
</tr>
<tr>
<td>Glucose utilization (fibroblasts)</td>
<td>0.02</td>
<td>Kronowskii et al. (1932)</td>
</tr>
<tr>
<td>Anaerobic glycolysis (yeast)</td>
<td>1</td>
<td>Nilsson et al. (1931)</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Bharoacetate</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Chloroacetate</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Effect</td>
<td>Relative concentrations or doses</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>----------------------------------</td>
<td>--------------------</td>
</tr>
<tr>
<td></td>
<td>Iodoacetate</td>
<td>Bromoacetate</td>
</tr>
<tr>
<td>Inhibition of yeast fermentation</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition of rat brain respiration</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>(minimal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition of E. coli respiration</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>(minimal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition of frog muscle respiration</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition of bacterial luminescence</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loss of excitability in frog muscle</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>(minimal)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inhibition of corn root growth</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>(50%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LD₅₅ for mice (oral)</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
inhibiting glycolysis (Lundsgaard, 1932), and have a low degree of toxicity (Morrison, 1946). The effect of the vicinal carboxylate or carboxamide group on the alkylating activity of the halogen atom is quite definite here, and even the propionate derivatives are mostly ineffective. Such relationships are, of course, not seen when a nonalkylating mechanism is responsible for the inhibition, as in the competition of various amides with acetaldehyde for alcohol dehydrogenase (Woronick, 1961). Here the size and the van der Waals’ forces of the halogen or aliphatic groups are more important in determining the potency of the inhibition.

**Esters of the Haloacetates**

The methyl and ethyl esters of bromoacetic and iodoacetic acids have been used to facilitate penetration, the early work apparently being based of the assumption that the esters are hydrolyzed to the active inhibitors within the cells. The important questions one must ask first are the following. Are these esters reactive with SH groups? How readily are the esters hydrolyzed? Are there enzymes within cells to catalyze this hydrolysis and, if so, how active are they? Is there evidence that the EM pathway can be blocked, or that the esters can induce rigor in muscle? There is no doubt that some of these esters are very potent metabolic inhibitors and toxic agents. Ethyl bromoacetate is very toxic and irritant to the skin and for this reason was used at the beginning of World War I as a war gas and studied at the Kaiser-Wilhelm Institute (Wachtel, 1920). Administration of 0.2 ml (1.4 mmoles) subcutaneously to cats leads to death within 1 hr, the toxic symptoms being referable to the central nervous system and death resulting from respiratory paralysis. The lethal dose for man by inhalation is 5000 ct.* Several of the esters have been used commercially as food preservatives, and are often potent bacteriostatic and fungistic agents; for example, several esters of bromoacetate inhibit the growth of *Torula utilis* 50% at concentrations around 0.003 mM (Hansen, 1956 a).

The pattern of enzyme inhibition by the esters of iodoacetate (Table 1-45) is not the same as for iodoacetate, and some SH enzymes are quite resistant to the esters. On the other hand, enzymes such as yeast hexokinase, heart succinate oxidase, milk xanthine oxidase, and yeast alcohol dehydrogenase are inhibited more strongly by the esters than by iodoacetate. It seems that 3-PGDH is not as sensitive to the esters. However, the results obtained on enzymes must be interpreted in light of the spontaneous hydrolysis of these esters. Bergmann and Shimoni (1953) found that the methyl and ethyl esters of the haloacetates are hydrolyzed rapidly, those

* This expresses exposure in terms of concentration and duration: $c = \text{concentration as cubic millimeters of ester per cubic meter of air, and } t = \text{exposure time in min.}$
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Ester</th>
<th>Concentration (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexokinase</td>
<td>Yeast</td>
<td>Ethyl</td>
<td>1.25</td>
<td>49</td>
<td>Bailey and Webb (1948)</td>
</tr>
<tr>
<td>Alcohol dehydrogenase</td>
<td>Yeast</td>
<td>Ethyl</td>
<td>0.17</td>
<td>85</td>
<td>Mackworth (1948)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.33</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>D-Amino acid oxidase</td>
<td>Rat liver</td>
<td>Methyl</td>
<td>1.34</td>
<td>0</td>
<td>Mackworth (1948)</td>
</tr>
<tr>
<td>Choline dehydrogenase</td>
<td>Horse serum</td>
<td>Ethyl</td>
<td>0.67</td>
<td>80</td>
<td>Mackworth (1948)</td>
</tr>
<tr>
<td>Cholinesterase</td>
<td>Horse serum</td>
<td>Ethyl</td>
<td>1.67</td>
<td>0</td>
<td>Mackworth (1948)</td>
</tr>
<tr>
<td>Cytochrome oxidase</td>
<td>Muscle</td>
<td>Methyl</td>
<td>0.67</td>
<td>0</td>
<td>Mackworth (1958)</td>
</tr>
<tr>
<td>Diaphorase</td>
<td>Muscle</td>
<td>Methyl</td>
<td>3.3</td>
<td>0</td>
<td>Mackworth (1948)</td>
</tr>
<tr>
<td>Glucose dehydrogenase</td>
<td>Ethyl</td>
<td>0.67</td>
<td></td>
<td></td>
<td>Mackworth (1948)</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>Muscle</td>
<td>Ethyl</td>
<td>0.2</td>
<td>50</td>
<td>Mackworth (1948)</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>Methyl</td>
<td>0.33</td>
<td>90</td>
<td></td>
<td>Mackworth (1948)</td>
</tr>
<tr>
<td>3-Phosphoglyceraldehyde dehydrogenase</td>
<td>Muscle</td>
<td>Ethyl</td>
<td>0.33</td>
<td>90</td>
<td>Mackworth (1948)</td>
</tr>
<tr>
<td>Pyruvate decarboxylase</td>
<td>Yeast</td>
<td>Ethyl</td>
<td>0.3</td>
<td>3</td>
<td>Stoppani et al. (1953)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.7</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3.5</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7.0</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>13.8</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Pyruvate oxidase</td>
<td>Yeast</td>
<td>Ethyl</td>
<td>0.67</td>
<td>20</td>
<td>Mackworth (1948)</td>
</tr>
<tr>
<td></td>
<td>E. coli</td>
<td>Methyl</td>
<td>0.62</td>
<td>85</td>
<td>Mackworth (1948)</td>
</tr>
<tr>
<td>Succinate oxidase</td>
<td>Heart</td>
<td>Ethyl</td>
<td>0.17</td>
<td>20</td>
<td>Mackworth (1948)</td>
</tr>
<tr>
<td>Urease</td>
<td>Jack bean</td>
<td>Ethyl</td>
<td>0.67</td>
<td>97</td>
<td>Mackworth (1948)</td>
</tr>
<tr>
<td>Xanthine oxidase</td>
<td>Milk</td>
<td>Methyl</td>
<td>2.5</td>
<td>50</td>
<td>Mackworth (1948)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.5</td>
<td>70</td>
<td>Mackworth (1948)</td>
</tr>
</tbody>
</table>
of iodoacetate the most rapidly and those of chloroacetate the least. Indeed, they could not measure the enzymic hydrolysis of ethyl iodoacetate because of the very rapid spontaneous hydrolysis. Wachtel (1920) noted upon dissolving ethyl bromoacetate in water that decomposition occurs, and considered the possibility of the toxic effects observed being due to the products. In the enzyme inhibition studies shown in Table 1-45 the incubations with the esters are usually 10-90 min, so one wonders how much of the ester is present at any time and how much of the inhibition is due to it. Furthermore, since the bromoacetate esters are hydrolyzed quite rapidly by acetylcholinesterase and liver esterase, this must be considered in work on cellular preparations (Bergmann and Shimoni, 1953). Shirk and Gertler (1958) studied the effect of bromoacetate esters on the growth of fungi, and found many of them to be very potent inhibitors. Ethyl bromoacetate inhibits *Aspergillus niger* growth 50% at 0.0025 mM and several others prevent growth completely at this concentration. These tests were made over a 96 hr period at 30°C so one would expect a good deal of hydrolysis. Bromoacetate itself is not nearly as potent an inhibitor, so one must assume that the esters either produce the inhibition directly or penetrate readily and release bromoacetate within the cell in much higher concentrations than would be obtained with bromoacetate alone. Very little seems to be known as to the reactivity of the esters with SH groups. Stoppani *et al.* (1953) stated that cysteine and dimercaprol provide complete protection of pyruvate decarboxylase against both iodoacetate and its ethyl ester, but this could mean only that the ester is hydrolyzed and acts through the formation of iodoacetate.

When we turn to the effects of these esters on metabolism, it is found that the actions are fundamentally not those of the unesterified haloacetates. Mackworth (1948) observed that ethyl iodoacetate inhibits the respiration of pigeon breast muscle and yeast very potently, in the case of yeast 50% inhibition being given by around 0.04 mM. Fleckenstein (1948) thoroughly investigated the effects on yeast and concluded that the esters are quite different in pattern of inhibition from the unesterified compounds. Whereas the latter inhibit glycolysis more than respiration, the esters are potent respiratory inhibitors (see accompanying tabulation). Ethyl iodo-

<table>
<thead>
<tr>
<th>Ethyl bromoacetate (mM)</th>
<th>% Inhibition of:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Respiration</td>
<td>Fermentation</td>
</tr>
<tr>
<td>0.0012</td>
<td>16</td>
<td>0</td>
</tr>
<tr>
<td>0.003</td>
<td>41</td>
<td>0</td>
</tr>
<tr>
<td>0.015</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>0.03</td>
<td>48</td>
<td>14</td>
</tr>
<tr>
<td>0.06</td>
<td>49</td>
<td>30</td>
</tr>
</tbody>
</table>
iodoacetate acts very similarly, whereas ethyl chloroacetate is considerably less potent, indicating the importance of the halogen atom. These esters are more potent respiratory inhibitors than cyanide, but it seems that in yeast only about 50% of the respiration is sensitive. The inhibition of respiration is rapid so that hydrolysis is probably not a factor here, but over several hours the inhibition disappears, which may be the result of hydrolysis.

The respiration of frog skin and muscle is less sensitive than that of yeast to ethyl bromoacetate, 50% inhibition being given by 0.12 mM in the former and 0.24 mM in the latter (Fleckenstein et al., 1950). However, one finds the same differential effects on respiration as in yeast. Fleckenstein believed that these esters must be classed as cycle inhibitors and demonstrated that several enzymes of the cycle are inhibited. Just et al. (1951) investigated the effect of bromoacetate esters of different chain length on yeast respiration and found even more potent inhibition than in the previous work (see accompanying tabulation). In the α-bromopropionate series

<table>
<thead>
<tr>
<th>Bromoacetate ester</th>
<th>Concentration for 50% inhibition (mM)</th>
<th>Relative potency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl</td>
<td>0.0036</td>
<td>17</td>
</tr>
<tr>
<td>Ethyl</td>
<td>0.0006</td>
<td>100</td>
</tr>
<tr>
<td>n-Propyl</td>
<td>0.0014</td>
<td>43</td>
</tr>
<tr>
<td>n-Butyl</td>
<td>0.0021</td>
<td>29</td>
</tr>
<tr>
<td>Iso-amyl</td>
<td>0.007</td>
<td>9</td>
</tr>
<tr>
<td>n-Hexyl</td>
<td>0.015</td>
<td>4</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.052</td>
<td>1</td>
</tr>
</tbody>
</table>

of esters, the ethyl ester is also the most potent inhibitor of respiration, but is about one fourth as active as the bromoacetate ester. All of these results indicate clearly that the esters can presumably act directly and not entirely by release of the haloacetates.

It is interesting that the amides and the esters act so differently, since this demonstrates that it is not simply a matter of the removal of the negatively charged carboxylate group. It is rather surprising that more work on the mechanisms of the respiratory inhibition has not been done, especially as these esters might well be valuable inhibitors for selective effects on certain pathways of metabolism. A few suggestions for those using such inhibitors may be made, inasmuch as the past studies have not usually included the data necessary to allow interpretation. Proper controls with the unsubstituted esters of acetate or propionate should be run to evaluate the role of the halogen atoms. The kinetics of the inhibitions should be followed closely, preferably with data on the hydrolytic rates for the esters.
The reversibility of the inhibitions should be tested since this will have bearing on whether an alkylating mechanism is involved or not.

**Halogenated Substances Which May Alkylate SH Groups**

A general review of the alkylating agents has been presented by Ross (1962) with the emphasis on compounds of possible use in cancer therapy. Some of these are halogenated substances which are reactive by virtue of the halogen atoms, but little is known of their effects on enzymes or metabolism. In this final section we shall consider only a few compounds which may be related in their actions to the haloacetates, and at least are often potent metabolic inhibitors. These substances are all potent vesicants and

![Chemical structures](image)

lachrymators, properties which Bacq (1941) attempted to correlate with inhibition of glycolysis. Fleckenstein *et al.* (1950), however, found them to be more inhibitory to respiration than glycolysis, behaving in general as the esters of bromoacetate, and classified them as cycle inhibitors. Fischer (1944) found that papain is inhibited 80–90% within 15–30 min by 0.5 mM chloropicrin, chloroacetone, and chloroacetophenone, all being somewhat more potent than iodoacetamide. Several enzymes were studied by Mackworth (1948) and some of the results are summarized in Table 1-46. She further found that the enzymes can generally be protected from these agents by cysteine or glutathione, indicating that reaction with SH groups occurs readily. Although these substances react with SH groups, one cannot yet attribute the inhibition of respiration or their vesicant action to this
Table 1-46

INHIBITION OF ENZYMES BY VARIOUS HALOGENATED COMPOUNDS

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Inhibitor</th>
<th>Concentration (mM)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Succinate oxidase</td>
<td>Chloropicrin</td>
<td>0.01</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.02</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.067</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>Chloroacetophenone</td>
<td>0.33</td>
<td>82</td>
</tr>
<tr>
<td>Choline dehydrogenase</td>
<td>Chloroacetophenone</td>
<td>0.4</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.67</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>Bromoacetophenone</td>
<td>0.22</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Bromobenzyl cyanide</td>
<td>0.4</td>
<td>80</td>
</tr>
<tr>
<td>Alcohol dehydrogenase</td>
<td>Chloroacetophenone</td>
<td>0.33</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Bromoacetophenone</td>
<td>0.33</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Bromobenzyl cyanide</td>
<td>0.33</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Chloropicin</td>
<td>0.33</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Iodoacetate</td>
<td>0.33</td>
<td>60</td>
</tr>
<tr>
<td>Pyruvate oxidase</td>
<td>Chloropicin</td>
<td>0.33</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Bromoacetophenone</td>
<td>0.33</td>
<td>95</td>
</tr>
<tr>
<td></td>
<td>Iodoacetate</td>
<td>0.62</td>
<td>0</td>
</tr>
<tr>
<td>3-Phosphoglyceraldehyde</td>
<td>Chloropicin</td>
<td>0.33</td>
<td>20</td>
</tr>
<tr>
<td>dehydrogenase</td>
<td>Chloroacetophenone</td>
<td>1</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Bromoacetophenone</td>
<td>0.33</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Bromobenzyl cyanide</td>
<td>0.5</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>Iodoacetate</td>
<td>0.2</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.33</td>
<td>90</td>
</tr>
<tr>
<td>Xanthine dehydrogenase</td>
<td>Chloropicin</td>
<td>0.83</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>Bromoacetophenone</td>
<td>0.42</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.6</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>Bromobenzyl cyanide</td>
<td>0.83</td>
<td>93</td>
</tr>
<tr>
<td>Cholinesterase</td>
<td>Chloropicin</td>
<td>2.74</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Bromoacetophenone</td>
<td>0.8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.74</td>
<td>60</td>
</tr>
<tr>
<td>Urease</td>
<td>Bromoacetophenone</td>
<td>1.67</td>
<td>80</td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>Bromoacetophenone</td>
<td>0.67</td>
<td>0</td>
</tr>
</tbody>
</table>

*In all cases the incubation times were 15-30 min. (From Mackworth, 1948.)*
mechanism. It may also be noted that chloroaceto phenone inhibits *E. coli* tryptophanase quite readily — 5% inhibition at 0.002 mM, 44% at 0.004 mM, and 73% at 0.02 mM — and that glutathione protects (Turner and Happold, 1960).

It is noteworthy that chloroacetone is more potent than iodoacetate in killing paramecia (Calcutt, 1950), and that chloropicrin is much more potent than iodoacetamide in preventing the contraction of actomyosin fibers induced by ATP (Godeaux, 1944). The extensive studies of A.F.W. Hughes (1950) on the antimitotic effects of chloroacetophenone in chick tissue cultures further indicate the potency of this compound. Very briefly, the following effects were observed: (1) a concentration of 0.33 mM allows the cells to enter prophase, but no spindle is formed and the chromosomes do not aggregate on the metaphase plate, (2) a concentration of 0.013-0.033 mM applied to cells in anaphase slows chromosomal movement and causes excessive surface bubbling, but allows fairly normal cleavage, (3) a concentration of 0.13 mM prevents the formation of the cleavage furrow, and (4) concentrations above 0.065 mM progressively distort cleavage patterns and retard nuclear reconstruction. It is unfortunate that so little is known of the mechanisms by which these halogenated substances act, inasmuch as some of them might be useful detectors of SH groups or other groups on enzymes. It would be valuable to have comparable data on the effects of the nonhalogenated compounds, or derivatives in which the halogen atoms are replaced by other groups, in order to be able to determine the importance of these halogen atoms.
Maleate and fumarate are isomeric, the former being the cis and the latter the trans form, and for this reason the differences in the actions on biological systems were studied early to demonstrate the influence of structure on activity. Maleic acid was discovered by Lassaigne in 1818, who prepared it by heating malic acid and named it paramalic acid; it was renamed maleic acid by Pelouze in 1834. The first report of its toxicity was made by Foderá (1894), who found that given intravenously to dogs at a dose of 1.94 g/kg (neutralized) it caused rapid death, whereas the same quantity of fumaric acid produced only a weak and nontoxic response. The greater toxicity of maleate relative to fumarate was confirmed during the next few years with whole plants and isolated plant tissues, algae, infusoria, copepods, and other organisms. It has also been observed that many tissues can readily metabolize fumarate but not maleate, as first reported by Maassen (1895) in the penicillia, and that under certain conditions fumarate may go to lactate whereas maleate is unable to do so. Ohta (1912) believed that maleate could be transformed to acetoacetate in the perfused dog liver, but it seems more likely that the observed increase in acetoacetate was due to the effect of the maleate on the metabolism of the liver. Lipschitz and Gottschalk (1921) showed that fumarate brought about an enzyme-catalyzed reduction of m-dinitrobenzene in ground muscle but that maleate had no effect, and Dakin (1922) established that incubation of fumarate with a muscle extract led to the appearance of malate, while no malate arose from maleate.

The first report of a truly inhibitory action of maleate on metabolism was by Thunberg (1911 a), who demonstrated a fairly potent depression of frog muscle respiration; indeed, it was the most potent inhibitor of all the many organic acids examined. This study was extended by Grönvall
(1924) to several tissues of various animals, in which fumarate stimulated the respiration and maleate depressed it at identical concentrations. When the competitive action of malonate against succinate oxidation became known, it was easy for Gözy and Szent-Györgyi (1934) to postulate without evidence that maleate is related to fumarate as malonate to succinate, in order to explain the inhibition of muscle respiration by maleate and other reported effects for which no data were given. The relationship between maleate and fumarate is, of course, entirely different from that between malonate and succinate, and it was soon shown by Morgan and Friedmann (1938 b) that the inhibition of metabolism and of several enzymes by maleate is due primarily to the ability to react with SH groups, and more recently Massey (1953 b) has shown fumarase to have a low affinity for maleate. The generally irreversible nature of the inhibition also points to a chemical reaction rather than a simple competitive effect due to its structural similarity to other dicarboxylic acids, although we shall see that competitive behavior is occasionally observed.

During the past 20 years maleate has been shown to exert some very interesting actions on biological systems, but it is difficult in any case to ascribe its action to a definite and specific inhibition of a particular enzyme. When a comparative estimate of the sensitivities of various enzymes to maleate can eventually be made, these problems may be solved. In fact, it is not clear if maleate actually occurs naturally in living tissues; it has usually been stated that it does not, even in certain plants and fruits in which all the related acids occur (e.g., Schmalfuss and Keitel, 1924). Copisarow (1936) obtained a substance from apples (named "blastokolin") which inhibited ripening and germination in certain fruits and potatoes in a manner similar to maleate, and thought that the natural inhibitor might be maleate. Maleic acid has been found in Portuguese wines to the extent of 1.5 g/liter, but this does not imply that it is present in the grapes (Correia and Sérgio, 1943). Marinov (1950) reported that maleate occurs in potato plants and increases during infection with mosaic virus. Maleate has not been certainly detected in animal tissues, but a thorough search with sensitive methods has not been done, and since various maleyl derivatives are now known to be intermediates in metabolism and new enzymes have been reported for the metabolism of maleate, it seems likely that at least low concentrations may be present (see page 313). Maleate is an interesting inhibitor and merits more serious investigation, particularly with respect to its renal actions and antimitotic potential.

CHEMICAL PROPERTIES

The physical and chemical properties of maleic acid differ markedly from those of fumaric acid, since the position of the carboxyl groups is a major
factor determining these properties. Some properties of these acids and their anions are compared in Table 2-1 for reference. The solubilities and ionization constants are particularly different and provide much useful information on the structure of the maleate ion. It is noteworthy that maleate usually chelates more strongly than fumarate with cations, this presumably being related to the closer intercarboxylate distance in maleate, which is not much greater than for malonate (3.28 Å). This complexing with cations must always be considered in using maleate as an inhibitor, especially at higher concentrations, since this alone may alter enzyme activity or tissue function.

<table>
<thead>
<tr>
<th>Property</th>
<th>Maleic acid</th>
<th>Fumaric acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solubility in water (g/100 g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10°C</td>
<td>32.6</td>
<td>0.96</td>
</tr>
<tr>
<td>37°C</td>
<td>52.1</td>
<td>0.71</td>
</tr>
<tr>
<td>80°C</td>
<td>75.0</td>
<td></td>
</tr>
<tr>
<td>Solubility in ether (g/100 g)</td>
<td>7.6</td>
<td>0.71</td>
</tr>
<tr>
<td>Solubility in benzene (g/100 g)</td>
<td>0.024</td>
<td>0.003</td>
</tr>
<tr>
<td>Partition ratio between water and methylisobutylketone</td>
<td>4.7</td>
<td>0.68</td>
</tr>
<tr>
<td>Melting point</td>
<td>131°C</td>
<td>287°C</td>
</tr>
<tr>
<td>Intercarboxylate distance (Å)</td>
<td>3.66</td>
<td>4.87</td>
</tr>
<tr>
<td>Ionization constants (37°, s = 0.15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$K_{a1}$</td>
<td>$2.14 \times 10^{-2}$</td>
<td>$1.41 \times 10^{-3}$</td>
</tr>
<tr>
<td>$pK_{a1}$</td>
<td>1.67</td>
<td>2.85</td>
</tr>
<tr>
<td>$K_{a2}$</td>
<td>$1.78 \times 10^{-6}$</td>
<td>$1.00 \times 10^{-4}$</td>
</tr>
<tr>
<td>$pK_{a2}$</td>
<td>5.75</td>
<td>4.00</td>
</tr>
<tr>
<td>$K_{a1}/K_{a2}$</td>
<td>12,000</td>
<td>14.1</td>
</tr>
<tr>
<td>Dissociation constants for complexes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$pK$ for Ca$^{++}$ complex (37°, s = 0.15)</td>
<td>1.20</td>
<td>0.58</td>
</tr>
<tr>
<td>$pK$ for Ba$^{++}$ complex (25°, s = 0)</td>
<td>2.26</td>
<td>1.59</td>
</tr>
<tr>
<td>$pK$ for Cu$^{++}$ complex (25°, s = 0)</td>
<td>3.90</td>
<td>2.51</td>
</tr>
<tr>
<td>(37°, s = 0.15)</td>
<td>3.06</td>
<td>1.67</td>
</tr>
<tr>
<td>$\Delta F$ for reduction to succinate (keal/mole)</td>
<td>$-29.8$</td>
<td>$-23.2$</td>
</tr>
<tr>
<td>Molar reduction potential at dropping mercury electrode (volts)</td>
<td>$-0.409$</td>
<td>$-0.414$</td>
</tr>
<tr>
<td>Polarographic half-wave potential in neutral solution (volts)</td>
<td>$-1.36$</td>
<td>$-1.58$</td>
</tr>
</tbody>
</table>
Ionization of Maleic Acid

The pKₐ of maleic acid (1.67) is much lower than that for fumaric (2.85) or succinic (3.95) acid; thus the first proton is dissociated very readily from maleic acid and the second proton with difficulty. These unique properties have stimulated many hypotheses for the mechanisms involved, and from these has come a better understanding of the structure of maleic acid which is of some importance in considerations of its action on enzymes. Electrostatic interactions between the cis carboxylate groups have been invoked to explain the aberrant pKₐ's. Certainly bringing the carboxylate groups closer together (as in the series of succinic-malonic-oxalic acids) decreases pKₐ, and the propinquity imposed by the cis form must play a role, but the groups in maleate are not closer than in malonate and so some additional factor must be considered. This was provided by the crystallographic study of Shahat (1952), who demonstrated that crystals of maleic acid are made up of layers of nearly flat molecules, the structure of each molecule being as represented in Fig. 2-1. Adjacent molecules in the layers are bound through hydrogen bonds, while the layers are held together by van der Waals' forces. The unequal distances of the C—O bonds are probably due to hydrogen bonding between the O atoms, this effectively making a seven-membered ring. It is interesting, in passing, that the C—C distances are approximately equivalent and correspond to around 25–30% double-bond character, indicating a fair degree of resonance. The hydrogen bond is a strong one and abnormally short (most are 2.7–2.8 Å). These observations stimulated the publication of six communications in 1953 on the role of hydrogen bonding in the ionization of maleic acid.

L. Hunter (1953) reasoned that if such a hydrogen bond were retained in ionizing solvents, it would tend to stabilize the H-maleate⁻ ion; the

![Fig. 2-1. Structural characteristics of maleic acid. (From Shahat, 1952.)](image-url)
affinity of the hydrogen-bonded carboxylate group for protons would be lessened and account for the low $pK_a$. This anion would also derive additional stability from resonance between structures (II) to (V), and this would diminish the tendency for loss of a second proton, accounting for the high $pK_a$. Such hydrogen bonding would, of course, not occur in fumaric acid. Davison (1953) believed, on the other hand, that the hydrogen bond would not survive competition from water molecules, and stated that preliminary infrared data showed no bands indicating hydrogen bonding in solution. However, Cardwell et al. (1953) found infrared bands suggestive of a hydrogen bond in H-maleate$, the bond being more symmetrical in the ion than in maleic acid, so that structure (VI) might be more appropriate. The dissociation of the first proton would cause the second proton to move into a more symmetrical position. McDaniel and Brown (1953) also provided evidence that the hydrogen bond may well be retained in solution. Nash (1953) felt that the presence of a hydrogen bond in H-maleate$ would decrease the symmetry and resonance of the molecule, and that this would oppose the formation of the hydrogen bond. Instead, he postulated that the proton exists within the molecule:
this being perhaps an instance of proton adsorption. Crawford (1953) argued that, although maleic acid in the crystal is quite planar, in solution one carboxyl group may not lie in the plane of the molecule, and resonance symmetry will be lost. Electrons will tend to be withdrawn from the non-planar carboxyl group and its acidity will be increased, accounting for the low pK_a. The ionization of the second carboxyl group being simultaneously depressed. This theory would assume a decreased stability of the undisassociated acid rather than an increased stability of the anion. Recent studies have minimized the importance of the hydrogen bonding. Although infrared data indicate that H-maleate^− is internally hydrogen bonded, while maleic acid is not, it was felt that the pK_a values can be accounted for adequately on the basis of electrostatic and dipole interactions (Dodd et al., 1961). Ionization enthalpies of dicarboxylic acids suggest that hydrogen bonding is not important when ΔpK_a is less than 4, but becomes progressively important at values greater than this; thus the contribution for maleic acid must be very small (Eberson and Wadsö, 1963). It is difficult to decide between these mechanisms and possibly several contribute significantly.

At pH's around neutrality there would be relatively more of the singly charged H-maleate^- anion than of the comparable anion of other dicarboxylic acids, and this might facilitate the penetration of maleate into cells if the monanion can enter. The ratio of the dianion to the monanion at pH 7.4 is 45, so that around 2% would exist as the monanion, whereas in the case of malonate only 0.7% would be in this form. On the other hand, if only the undisassociated acid can penetrate, very little will enter the cell since the concentration of this form at pH 7.4 would be near 4 × 10^-7 mM when the total concentration of maleate is 10 mM. We may note that the pH of a 100 mM solution of maleic acid is 1.90 and of sodium hydrogen maleate is 4.18, so that adjustment of the pH is required before use (Kolthoff and Tekelenburg, 1927).

**Cis-Trans Isomerization**

The ΔF for the reaction maleic acid → fumaric acid was determined by Parks and Huffman (1930) to be −6.6 kcal/mole at 25°, but more recent studies have pointed to a somewhat lower value. Thus Davies and Evans (1956) determined the equilibrium constant, K_eq = (fumaric acid)/(maleic acid), at temperatures between 80° and 130°, and from these data the following thermodynamic parameters may be obtained: ΔH = −8.0 kcal/mole, ΔS = −17.1 cal/mole/degree, and ΔF (37°) = −2.4 kcal/mole. Extrapolation of their data to 37° gives a K_eq around 47, so that at equilibrium there would be approximately 2% maleic acid and 98% fumaric acid. The K_eq for the dianions, i.e., (fumarate)/(maleate), would be larger and perhaps around 175, taking into account the differences in ionization.
The isomerization of maleic acid to fumaric acid occurs at a very slow rate in aqueous solution. Kailan (1919) kept a solution of maleic acid in the dark and at room temperature for 5 years, and found that only 4% had been transformed into fumaric acid. Davies and Evans (1956) studied the kinetics of the reaction but their work was done at elevated temperatures. However, even at 120° it requires 10–20 hr for a 25% conversion of maleic to fumaric acid. An activation energy of 21 kcal/mole for the uncatalyzed reaction was obtained, which is of the same order of magnitude as the 25 kcal/mole activation energy found for the isomerization of dimethylmaleate (Davies and Evans, 1955). The isomerization involves a heterolytic mechanism and is mainly second order. The reaction is accelerated by increase in temperature, ultraviolet irradiation, acid, and various catalysts. One may conclude that, under ordinary conditions, solutions of maleate are quite stable.

Thiols are able to catalyze many cis-trans isomerizations by means of thiyl radicals. Morgan and Friedmann (1938 a) observed that when maleate is allowed to react with various thiols at 37° and pH 7.4, a certain amount of fumarate is formed. Olefinic double bonds and thiols which can react to form addition products are apparently necessary for this catalysis, since cinnamate is not isomerized by glutathione. Knox and Edwards (1954) also found that maleylacetooacetate is isomerized to fumarylacetooacetate by glutathione, a reaction of possible importance in the metabolism of homogentisate. Waley (1962) postulated that the R–S⁻ anion may be the reactive species, the intermediate formed being a carbanion. The intermediate can, in the case of maleate, either be converted into the trans form or pick up a proton to form the addition compound. The relative rates will determine how much maleate will be isomerized to fumarate. Such reactions must be borne in mind when maleate is in the presence of thiols, and may be important in the metabolism of maleate in the tissues.

**Purification and Determination**

Maleic acid obtained commercially is usually not sufficiently pure for most work and may contain some fumaric and malic acids. It may be dissolved in warm water, treated with activated charcoal, and crystallized by cooling. Two crystallizations usually suffice to give a satisfactory preparation with a constant melting point. It may also be crystallized from acetone, as was done by Shahat (1952) for his crystallographic work. It has been stated that the only method giving pure maleic acid, free from fumaric acid, is to dissolve several times redistilled maleic anhydride in distilled water and crystallize the maleic acid by concentration in vacuo (Parks and Huffman, 1930) and this is perhaps the preferred method. Maleic acid is probably best detected and determined in a mixture of acids obtained by fractionation of biological material by two-dimensional chromatography.
Detection in simpler mixtures by polarography is also possible (Kolthoff and Lingane, 1952).

**General Reactions of Maleate**

Most of the biologically important reactions of maleate involve addition to the double bond. (1) *Addition of water* to form malate occurs very slowly at physiological temperatures and the uncatalyzed reaction need not be considered in work with maleate. Even at 140°, only 25% of maleate is converted to malate in 10 hr (Weiss and Downs, 1922), and a 2.1 M solution of maleic acid allowed to stand for 10 hr at 130° contains 30% fumarate and 10% malate (Davies and Evans, 1956). The possibility of enzymic conversion to malate will be discussed later (page 313). (2) *Addition of amines* to form substituted aspartate does not occur readily and the reaction:

\[ \text{R—NH}_2 + \text{CH—COO}^- \rightarrow \text{R—NH—CH—COO}^- \]

is very slow even at elevated temperatures. (3) *Addition of pyrroles* may proceed readily, the reaction with α-methylpyrrole

\[ \text{CH—COO}^- + \text{H}_3\text{C}—\text{NH—H—CH—COO}^- \rightarrow \text{CH}_2—\text{COO}^- \]

occurring at room temperature, especially in acid solutions, to form 2-methyl-5-pyrrolesuccinate. Such a reaction might be of importance biologically if it occurs with other pyrroles or heterocyclic compounds. (4) *Hydrogenation* usually is rather difficult; e.g., with Raney nickel catalyst it requires 100° and 2500 lb/square inch pressure, but with platinum catalyst it may occur measurably at ordinary temperatures. There is no evidence that succinate is formed directly from maleate under physiological conditions.

Maleate can be oxidized by permanganate and other strong oxidants to meso-tartrate, and possibly an oxidation of this type with the introduction of two hydroxyl groups must be considered biologically. Neuberg and Rubin (1914) believed acetaldehyde to be an important metabolic product and showed that it could be formed from maleate and fumarate by treatment with hydrogen peroxide and Fe++. Decarboxylation of maleate is not common under ordinary conditions, but ultraviolet irradiation for even short periods produces significant amounts of acrylate and CO₂. The book by Flett and Gardner (1952) should be consulted for the chemistry of maleate and its derivatives.
Reactions with Thiols

Inasmuch as maleate is often considered to act as an SH reagent to inhibit certain enzymes, it is necessary to examine the reaction with thiols in some detail. Morgan and Friedmann (1938 a) observed that cysteine, glutathione, and thiolacetate lose their SH groups when incubated with maleate at pH 7.4 and 37°, aerobically and anaerobically, whereas incubation with fumarate is ineffective. When maleate and thiolacetate are mixed in equimolar concentrations (20 mM), half the SH groups disappear in around 6 hr; as mentioned previously, fumarate is also formed and thus the reaction with the SH groups does not go to completion. When maleate is 80 mM and thiolacetate 20 mM, half reaction occurs in 80 min and complete reaction in several hours. The reaction with glutathione is very similar, but with cysteine the rate is greater and the reaction is more likely to go to completion. The products of the reactions were isolated, in the case of cysteine in 93.5% yield. The tricarboxylic S-cysteinosuccinic acid was crystallized and characterized by Morgan and Friedmann (1938 c). It was pointed out that this substance might be found in hydrolyzates of proteins treated with maleate, if reaction with protein cysteine occurs. The reaction of maleate with thiolacetate was also investigated by Weller et al. (1957). The addition of thiols to double bonds may involve a carbonium ion intermediate:

\[
\begin{align*}
-\text{CH} & \quad + \text{H}^+ \quad \rightarrow \quad -\text{CH}_2 \\
-\text{CH} & \quad + \text{S} \quad \rightarrow \quad -\text{CH}_2 \\
-\text{CH} & \quad + \text{S} \quad \rightarrow \quad -\text{CH}_2
\end{align*}
\]

and thus the rate will depend strongly on the pH, but this has not been investigated experimentally in the case of maleate. It may be noted that a variety of related compounds react with thiols to varying degrees; these include maleurate, \(N\)-ethylmaleimide, and maleic hydrazide.

Reactions with Proteins

When rabbit muscle and liver protein preparations are incubated with 80 mM maleate at pH 7.4 and 37°, there is a gradual disappearance of the SH groups, as determined by the nitroprusside reaction, and by 20 hr no SH groups remain (Morgan and Friedmann, 1938 b). Denatured ovalbumin presumably reacts similarly. Unfortunately no kinetic data were presented so the initial rates of reaction are unknown. Bovine serumalbumin forms complexes with various sulfonated azo dyes (e.g. methyl orange) through electrostatic forces between the sulfonate groups and quaternary \(N^+\) protein groups, with contributions from van der Waals' forces. Certain organic acid anions are able to compete with the dyes for the positively charged protein groups (Klotz, 1946). Maleate displaces methyl orange and
azosulfathiazole at pH 5.82-6.67 but is ineffective at pH 8.64. This competition is presumably not due to reaction of the maleate with protein SH groups but to relatively nonspecific electrostatic interactions, and yet the pH data are not entirely consistent with this explanation. It is interesting that succinate is a poor displacer, which was attributed to a lesser degree of ionization, but at the pH’s used this is not the case. In any event, these results demonstrate that maleate can interact significantly with serum albumin.

**INHIBITION OF ENZYMES**

The effects of maleate on enzymes are summarized in Table 2-2. These data are often of little quantitative significance since the complete experimental conditions, especially the incubation interval, are not given. Maleate frequently inhibits slowly so that the time of contact with the enzyme is very important. Morgan and Friedmann (1938 b) found, for example, that papain is inhibited 32% after 2 hr and 69% after 16 hr by 80 mM maleate. The results on succinate dehydrogenase and oxidase in the table are quite variable and part of this is due to quite different conditions and incubation durations. Thus, Hopkins et al. (1938) obtained their inhibition of succinate dehydrogenase by allowing the enzyme to react with maleate overnight, whereas others who report little or no inhibition have usually used more conventional intervals. However, perusal of the table provides certain conclusions, particularly that maleate does not inhibit some enzymes which appear to be typical SH enzymes, e.g., homogentisate oxidase (which is inhibited 89% by 1 mM p-MB), maleate dehydrogenase (decarboxylating) (which is inhibited 87% by 1 mM PM), β-amylase (which is inhibited 73% by 0.001 mM PM), isocitrate dehydrogenase (which is inhibited 100% by 0.04 mM p-MB), and protein phosphokinase (inhibited 45% by 0.1 mM p-MB). Succinate dehydrogenase is inhibited quite weakly by maleate, which is surprising in view of the probability that an SH group occurs in the active site (Potter and DuBois, 1943) and that the cationic sites might be expected to bind maleate in a position in which reaction of the SH with the double bond would occur, but it is likely that the configuration of maleate is such that the double bond may be hindered from approaching the SH group, as discussed in Chapter II-1 (page 34). It is interesting that bromelain and solanain are scarcely affected by maleate, whereas asclepain is strongly inhibited; yet all are SH enzymes and well inhibited by Hg++ and iodine. Apparently only certain SH groups on enzymes are subject to attack by maleate, so that maleate is certainly not as general an inhibitor of SH enzymes as most SH reagents. The steric and electrostatic requirements for maleate are perhaps more rigid. When maleate approaches an enzyme surface it may be either repelled by negatively charged groups or attracted by positively charged groups, but in
the latter case the molecule may be so oriented that the double bond is directed away from the surface. It is likely that only those SH groups can react when there are no charged groups in the immediate environment, or when the pattern of charged groups allows maleate to assume the correct orientation.

Let us summarize some of the possible ways in which maleate could inhibit enzymes. (1) Reaction with SH groups. (2) Competition with anionic substrates. These two mechanisms will be discussed in greater detail. (3) Chelation of activating cations. Although maleate does not complex with the common cations as readily as malonate, it is often used in such high concentrations that depletion of a metal cofactor could account for the inhibition. The decarboxylating maleate dehydrogenase of pigeon liver is inhibited by maleate more potently when the Mn++ concentration is reduced, indicating that at least part of the inhibition may be due to chelation of the Mn++ (Stickland, 1959 b). (4) Chelation of enzyme-bound metal ions. Possibly maleate can complex with metal cations which are associated tightly with the enzyme active center, especially in view of the positions of the carboxylate groups, although no clear-cut example of this is known. It may be that the potent inhibition of the oxidase activity of the copper-containing ceruloplasmin by maleate is the result of metal ion complexing, since fumarate and succinate are inactive (Curzon, 1960). However, the reaction with SH groups cannot be eliminated, since Hg++ inhibits 50% at 0.01 mM. (5) Reaction with substrate. The inhibition of the hydrolysis of ovalbumin by pepsin observed with maleate was attributed by Crippa and Maffei (1940) to reaction of the maleate with the ovalbumin rather than with the pepsin. (6) Nonspecific ionic or ionic strength effects. When maleate is used at higher concentrations (especially above 10 mM), one must consider effects on the enzyme mediated through changes in the ionic strength or by the cation accompanying the maleate. The inhibition of urease by maleate and other anions at concentrations from 50 to 139 mM was interpreted in terms of an increase in ionic strength by Kistiakowsky et al. (1952), with additional more specific contributions in certain cases. Native aspartate transcarbamylase is stimulated by 0.5 mM maleate and inhibited at higher concentrations; the native enzyme is a tetramer and possibly maleate weakens this structure, but by what mechanism is unknown (Gerhart and Pardee, 1964).

Maleate is one of the least dependable detectors of enzyme SH groups. However, it is just this property which might make it a useful inhibitor if a certain enzyme or group of enzymes is attacked specifically. Although maleate has not been tested on many enzymes, the available results point to two important types of enzyme as being especially sensitive to maleate, the α-keto acid oxidases and the transaminases, and certainly many of the metabolic effects of maleate can be satisfactorily explained on the basis of
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Maleate (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aconitase</td>
<td>Pigeon muscle</td>
<td>52</td>
<td>0</td>
<td>Breusch and Keskin (1944)</td>
</tr>
<tr>
<td>Alcohol dehydrogenase</td>
<td>Horse liver</td>
<td>—</td>
<td>40</td>
<td>Lutvak-Mann (1938)</td>
</tr>
<tr>
<td>d-Amino acid oxidase</td>
<td>Lamb kidney</td>
<td>3</td>
<td>0</td>
<td>Frisell et al., (1956)</td>
</tr>
<tr>
<td>α-Amylase</td>
<td>Bacillus subtilis</td>
<td>10</td>
<td>19</td>
<td>DiCarlo and Redfern (1947)</td>
</tr>
<tr>
<td>β-Amylase</td>
<td>Wheat</td>
<td>10</td>
<td>0</td>
<td>Ghosh (1958)</td>
</tr>
<tr>
<td>Arylsulfatase</td>
<td>Charonia lampas liver</td>
<td>2</td>
<td>28</td>
<td>Takahashi (1960 a)</td>
</tr>
<tr>
<td>Asclepain m</td>
<td>Milkweed latex</td>
<td>30</td>
<td>92</td>
<td>Greenberg and Winnick (1940)</td>
</tr>
<tr>
<td>Aspartase</td>
<td>Propionibacterium peterssonii</td>
<td>100</td>
<td>0</td>
<td>Ellfolk (1954)</td>
</tr>
<tr>
<td>Aspartate transcarbamylase</td>
<td>E. coli</td>
<td></td>
<td>$K_i = 0.7$</td>
<td>Gerhart and Pardee (1964)</td>
</tr>
<tr>
<td>Bromelain</td>
<td>Pineapple</td>
<td>30</td>
<td>0</td>
<td>Greenberg and Winnick (1940)</td>
</tr>
<tr>
<td>Cellulose polysulfatase</td>
<td>Charonia lampas liver</td>
<td>2</td>
<td>23</td>
<td>Takahashi (1960 b)</td>
</tr>
<tr>
<td>Ceruloplasmin</td>
<td>Human plasma</td>
<td>0.01</td>
<td>23</td>
<td>Curzon (1960)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05</td>
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<tr>
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<td>Human plasma</td>
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<td>12</td>
<td>Mounter and Whittaker (1953)</td>
</tr>
<tr>
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</tr>
<tr>
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<td>4</td>
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</tr>
<tr>
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<td>59</td>
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<tr>
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<td>100</td>
<td>Crewther (1953)</td>
</tr>
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<td>1</td>
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</tr>
<tr>
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<td>10</td>
<td>10</td>
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<tr>
<td>Glucose dehydrogenase</td>
<td>Beef liver</td>
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<td>14</td>
<td>Nakamura (1954)</td>
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<tr>
<td>Glutamate decarboxylase</td>
<td><em>Cucurbita moschata</em></td>
<td>2</td>
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<td>Ohno and Okunuki (1962)</td>
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<td>L-Glutamate dehydrogenase</td>
<td>Beef liver</td>
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<td>Caughey <em>et al.</em> (1957)</td>
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<td>0</td>
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<tr>
<td>β-Glycerophosphatase</td>
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<td>Nigam <em>et al.</em> (1959)</td>
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<td>Glyoxalase</td>
<td>Beef liver</td>
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<td>75</td>
<td>Morgan and Friedmann (1938 b)</td>
</tr>
<tr>
<td>Homogentisate oxidase</td>
<td>Rat liver</td>
<td>50</td>
<td>2</td>
<td>Crandall (1955)</td>
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<td>Isocitrate dehydrogenase</td>
<td>Pig heart</td>
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<td>0</td>
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<td>29</td>
<td>Angielski and Rogulski (1962)</td>
</tr>
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<td></td>
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<td>76</td>
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<td>Angielski and Rogulski (1962)</td>
</tr>
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<td></td>
<td>Rat liver</td>
<td>0.5</td>
<td>0</td>
<td>Angielski and Rogulski (1962)</td>
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<td></td>
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<td>10</td>
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</tr>
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<td>Lactate dehydrogenase</td>
<td>Rabbit muscle</td>
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<td>14</td>
<td>Hopkins <em>et al.</em> (1938)</td>
</tr>
<tr>
<td>Lipase</td>
<td>Pig pancreas</td>
<td>20</td>
<td>Stim</td>
<td>Yamamoto (1951)</td>
</tr>
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<td>Enzyme</td>
<td>Source</td>
<td>Maleate (mM)</td>
<td>% Inhibition</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>-----------------------------</td>
<td>--------------</td>
<td>--------------</td>
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<td>Nossal (1952)</td>
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<td>Malate dehydrogenase</td>
<td>Pigeon liver</td>
<td>20</td>
<td>50</td>
<td>Scholefeld (1955)</td>
</tr>
<tr>
<td>(decarboxylating)</td>
<td>Pig heart</td>
<td>30</td>
<td>0</td>
<td>Green (1936)</td>
</tr>
<tr>
<td></td>
<td>Rat kidney</td>
<td>5</td>
<td>80</td>
<td>Angielski and Rogulski (1962)</td>
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<tr>
<td></td>
<td>Pigeon liver</td>
<td>10</td>
<td>21</td>
<td>Stickland (1959 b)</td>
</tr>
<tr>
<td></td>
<td>(decarboxylating)</td>
<td></td>
<td></td>
<td>van Heyningen and Pirie (1953)</td>
</tr>
<tr>
<td></td>
<td>Beef lens</td>
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<td>0</td>
<td></td>
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<tr>
<td>Mucinase</td>
<td><em>Clostridium perfringens</em></td>
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<td>0</td>
<td>Robertson <em>et al.</em> (1940)</td>
</tr>
<tr>
<td>Myrosulfatase</td>
<td><em>Charonia lampedas</em> liver</td>
<td>2</td>
<td>28</td>
<td>Takahashi (1960 a)</td>
</tr>
<tr>
<td>Papain</td>
<td>Papaya latex</td>
<td>80</td>
<td>40</td>
<td>Morgan and Friedmann (1938 b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.4</td>
<td>34</td>
<td>Ganapathy and Sastri (1939)</td>
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<td></td>
<td></td>
<td>30</td>
<td>90</td>
<td>Greenberg and Winnick (1940)</td>
</tr>
<tr>
<td>Pepsin</td>
<td>Beef stomach</td>
<td>—</td>
<td>Inh</td>
<td>Crippa and Maffei (1940)</td>
</tr>
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<td>Pig kidney</td>
<td>100</td>
<td>52</td>
<td>Knox and Pitt (1957)</td>
</tr>
<tr>
<td>tautomerase *</td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>Human prostate</td>
<td>10</td>
<td>18</td>
<td>Anagnostopoulos (1953 a)</td>
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<td>Phosphatase (acid)</td>
<td>Human prostate</td>
<td>10</td>
<td>0</td>
<td>Nigam <em>et al.</em> (1959)</td>
</tr>
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<td>Phosphofructokinase</td>
<td>Sheep brain</td>
<td></td>
<td></td>
<td>Passonneau and Lowry (1963)</td>
</tr>
<tr>
<td></td>
<td><em>Charonia lammedas</em> liver</td>
<td>2</td>
<td>47</td>
<td>Takahashi (1960 b)</td>
</tr>
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<td>Polysaccharase</td>
<td><em>Charonia lammedas</em> liver</td>
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<td></td>
<td></td>
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<tr>
<td>Protein phosphokinase</td>
<td>Rabbit mammary gland</td>
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<td>Stim 5</td>
<td>Sundararajan <em>et al.</em> (1960)</td>
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<td>Dhar and Bose (1962)</td>
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<td>Yeast</td>
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<td>0</td>
<td>Kuhn and Beinert (1947)</td>
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<td>Pyruvate dehydrogenase</td>
<td>Pigeon brain</td>
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<td>34</td>
<td>Peters and Wakelin (1946)</td>
</tr>
<tr>
<td>Enzyme</td>
<td>Source</td>
<td>Ki (M)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>---------------</td>
<td>--------</td>
<td>------------------------------------------</td>
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<td>Pigeon brain</td>
<td>0.12</td>
<td>Peters and Wakelin (1946)</td>
<td></td>
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<td>Zittle (1946)</td>
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<td>Solanain</td>
<td>Horse nettle</td>
<td>30</td>
<td>Greenberg and Winnick (1940)</td>
<td></td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>Rabbit muscle</td>
<td>100</td>
<td>Hopkins et al. (1938)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rabbit muscle</td>
<td>80</td>
<td>Morgan and Friedmann (1938)</td>
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<td></td>
<td>Heart</td>
<td>K_i = 6</td>
<td>Dervartanian and Veeger (1962)</td>
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<td>Kidney</td>
<td>12.5</td>
<td>Takeya et al. (1953)</td>
<td></td>
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<td>Succinate oxidase</td>
<td>Pigeon brain</td>
<td>16.5</td>
<td>Peters and Wakelin (1946)</td>
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<tr>
<td></td>
<td>(+ cytochrome c)</td>
<td>16.5</td>
<td>21</td>
<td></td>
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<tr>
<td></td>
<td>Rat liver</td>
<td>40</td>
<td>Potter and DuBois (1943)</td>
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<td>5</td>
<td>Angielski and Rogulski (1962)</td>
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<tr>
<td></td>
<td>Rat heart</td>
<td>1</td>
<td>Montgomery and Webb (1956)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transaminases</td>
<td>Oat leaves</td>
<td>2.5</td>
<td>Fromageot and Patino-Bun (1961)</td>
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<td>Cysteinesulfinate:</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>α-ketoglutarate</td>
<td></td>
<td>10</td>
<td></td>
<td></td>
</tr>
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<td>Glutamate:oxalacetate</td>
<td>Rat liver</td>
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<td>Goldstone and Adams (1962)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pig heart</td>
<td>40</td>
<td>Jenkins et al. (1959)</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>Pig heart</td>
<td>5</td>
<td>Velick and Vavra (1962)</td>
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<td></td>
<td></td>
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<tr>
<td>Glycine:α-ketoglutarate</td>
<td>Rat liver</td>
<td>5</td>
<td>Nakada (1964)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kynurenine:α-ketoglutarate</td>
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<td>6</td>
<td>Mason (1959)</td>
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<td>Trehalase</td>
<td>Wax moth larvae</td>
<td>50</td>
<td>Kalf and Rieder (1958)</td>
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<td>Mushroom</td>
<td>20</td>
<td>Krueger (1955)</td>
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</tr>
<tr>
<td>Urease</td>
<td>Jack beans</td>
<td>87</td>
<td>Kistiaxowsky et al. (1952)</td>
<td></td>
</tr>
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</table>
these inhibitions. Peters and Wakelin (1946) first demonstrated that pyruvate oxidation is much more readily inhibited than succinate oxidase, the enzyme to which most attention had been previously paid. Furthermore, they demonstrated that the inhibition is not due to reaction of the maleate with low molecular weight thiols, since the dialyzed enzyme is also well inhibited. A 34% inhibition of pyruvate dehydrogenase by 0.04 mM and a 50% inhibition of the oxidase by 0.12 mM maleate are indicative of a marked susceptibility of this system. The oxidation of pyruvate by rat heart mitochondria is not as sensitive to maleate (see accompanying tabulation), but is more sensitive than the oxidations of malate, fumarate, isocitrate, and succinate (Montgomery and Webb, 1956 b). If the reaction of the maleate is with coenzyme A or lipoate, the \( \alpha \)-ketoglutarate oxidase should also be inhibited, and the results of Angielski and Rogulski (1962) indicate about the same sensitivity (Table 2-2) as the heart pyruvate oxidase. The fact that pyruvate decarboxylase seems to be insensitive to maleate would substantiate such a site of action.

The inhibition of pyruvate oxidation by heart mitochondria is always greater when fumarate is used as the source of oxalacetate than when maleate is used; the results in the tabulation above are from experiments with maleate, but when fumarate is used the inhibitions are some 10–20% higher. This could mean that some action is exerted on fumarase. Massey (1953 b) determined that \( K_i \) is 11 mM for the inhibition of pig heart fumarase by maleate; this is presumably a competition with fumarate rather than a reaction with SH groups. The inhibition of fumarase by 5 mM maleate in the presence of 5 mM fumarate (the concentration used in the mitochondrial work) can be calculated from the data of Massey to be around 11% at pH 6.35; at pH 6.8 the inhibition would be somewhat less since the affinity of the enzyme for maleate decreases above pH 6.4. Thus the inhibition of fumarase could contribute slightly at the highest concentration, assuming the results on crystalline fumarase can be applied to mitochondria, but could not be very important in the over-all affects on the cycle.

If the inhibition of an enzyme in due to reactions of its SH groups with maleate, the inhibition should be irreversible, but this has not been tested
in most cases where such a mechanism is the most likely. Greenberg and Winnick (1940) reported that the inhibitions of papain and asclepain by maleate can be to a large extent reversed by addition of cyanide. Since one might expect an SH reaction with these enzymes, the reversal is difficult to explain. They postulated that maleate oxidizes the enzymes, being simultaneously reduced to succinate, and that cyanide reactivates by restoration of the normal SH groups. However, it is thermodynamically unlikely that the reaction

Maleate + 2 SH → S—S + succinate

would occur, and furthermore in the studies in which maleate and various thiols have been allowed to react, no succinate has been reported. Since the reactivation by cyanide was not complete, it is possible that when maleate reacts with a certain SH group on the enzyme, secondary changes involving oxidation of other SH groups occur, these latter changes being reversible. One must admit that the concept of maleate as an SH reagent in enzyme inhibition is in no case on a solid basis, and there is much need for SH titration studies with pure enzymes exposed to this inhibitor.

Turning to the competitive mechanisms of inhibition, we are on much more solid, although often less interesting, ground. One would anticipate maleate to have some affinity for certain enzyme active sites normally reacting with other dicarboxylates. Competitive inhibition by maleate has been established for fumarase (Massey, 1953 b), cysteine desulfurase (Fromageot and Patino-Bun, 1961), transaminases (Jenkins et al., 1959; Velick and Vavra, 1962), and possibly β-glycerophosphatase (Nigam et al., 1959). In the case of transaminase, maleate is competitive with respect to both amino and keto acids, the inhibition is reversible by dialysis, and reaction of maleate with the enzyme can be followed spectrophotometrically. The results with succinate dehydrogenase have been so variable that it is difficult to characterize the mechanism of inhibition. In the early work of Hopkins et al. (1938) it was assumed that SH group reaction occurred because of the slow rate of inhibition, and this may well be true under their conditions. However, Dervartanian and Veeger (1962) found the inhibition to be competitive with \( K_i = 6 \text{ mM} \), and maleate to produce ultraviolet absorption changes similar to those observed when the enzyme is treated with fumarate or malonate. On the other hand, Hellerman et al. (1960) could detect no inhibition of the soluble succinate dehydrogenase from pig heart mitochondria, and in our work with rat heart mitochondria (Montgomery and Webb, 1956 b) we invariably found a stimulation of succinate oxidation by maleate at 1–5 mM. Certain enzymes which are inhibited by most dicarboxylates, such as aspartase (Ellfolk, 1954) and L-glutamate dehydrogenase (Caughey et al., 1957), are not inhibited by maleate, indicating the importance of the steric arrangement of the carboxylate groups.
In both instances, fumarate inhibits fairly potently. The fact that maleate inhibits the oxidation of several cycle substrates but has little effect on succinate in kidney mitochondria led Angielski and Rogulski (1962) to suggest that the inhibition may be on the transfer of electrons to or from NAD. No direct evidence was presented and, since there appears to be no studies of the effect of maleate on enzymes oxidizing NADH, any acceptance of this hypothesis must be tentative.

**EFFECTS ON RESPIRATION AND GLYCOLYSIS**

The respiratory response to maleate is frequently complicated by the metabolic utilization of the maleate by the tissue (see page 311) and in most cases this factor cannot be quantitatively evaluated. Respiration often is stimulated in plant tissues where metabolism of maleate has been demonstrated, but in animal tissues depression of respiration is the rule, although there is no way of telling if such depression is modified by some utilization of maleate. Fumarate, on the other hand, almost invariably accelerates the endogenous respiration of all tissues; the marked difference between fumarate and maleate is best seen in work with animal tissues (Grönvall, 1924). From the limited data available, it appears that maleate is as effective as fumarate, or more so, in stimulating root respiration (Lundegårdh, 1944; Biber and Farbman, 1951).

The effects of maleate on the respiratory quotient (Table 2-3) are equivocal, inasmuch as Thunberg (1911a) found a consistent decrease at low concentrations and an increase at very high concentrations in frog muscle, whereas Grönvall (1924) found rather small effects in a variety of tissues at a high concentration (50 mM), although some increase was most commonly observed. The fall in R. Q. with low maleate concentrations might be due to an inhibition of carbohydrate utilization and indeed a suppression of glucose oxidation has been observed in spermatozoa (Lardy and Phillips, 1943a) and *Azotobacter* (Lineweaver, 1933). Maleate also inhibits the fermentation of glucose by yeast (Jung and Müller, 1922). The respiration of brain slices is inhibited most potently by maleate when glucose is the substrate, and relatively little in the presence of the cycle substrates (Weil-Malherbe, 1938). Thus one might conclude that maleate is able to reduce the utilization of glucose or glycogen. But there is one metabolic response to maleate which, at least superficially, does not fit into this picture; aerobic glycolysis as measured by lactate formation is markedly stimulated, as shown in brain by Weil-Malherbe (1938) and in spermatozoa by Lardy and Phillips (1943b). This also occurs in embryo tissue, heart, spleen, and Jensen sarcoma, and the stimulation may amount to as much as 100–200%. The effects of maleate on the respiration and aerobic glycolysis of guinea pig brain slices are compared in Fig. 2-2; it is evident
that as respiration is depressed, lactate formation is accelerated. This cannot be attributed to an action on the EM pathway, since anaerobic glycolysis is depressed, or in the brain slices is unaffected. The most obvious explanation is a primary inhibition of oxidative processes by maleate and, since pyruvate and α-ketoglutarate oxidations have been shown

![Graph of Effects on Respiration and Glycolysis](image)

Fig. 2-2. Effects of maleate on the respiration and aerobic glycolysis of guinea pig brain slices. Glucose was present in all, and pyruvate or glutamate was added as indicated. (From Weil-Malherbe, 1938.)

to be relatively sensitive to maleate, a depression of the operation of the cycle leading to accelerated glycolysis is suggested. However, the effects of various substrates on the response of the brain slices to maleate are puzzling (Fig. 2-2). In the presence of pyruvate, for example, there is relatively little inhibition of respiration (10%) by 20 mM maleate, and
### Table 2-3

**Effects of Maleate on the Respiration and Respiratory Quotient of Tissues**

<table>
<thead>
<tr>
<th>Organism and preparation</th>
<th>Maleate (mM)</th>
<th>Interval (hr)</th>
<th>% Inhibition of respiration</th>
<th>R. Q.</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Brucella abortus</em> suspension</td>
<td>6.7</td>
<td>—</td>
<td>0</td>
<td></td>
<td>Gerhardt et al. (1950)</td>
</tr>
<tr>
<td><em>Rhizobium</em> root nodule bacteria</td>
<td>16.7</td>
<td>—</td>
<td>Stim 320</td>
<td></td>
<td>Burris and Wilson (1939)</td>
</tr>
<tr>
<td><em>Avena</em> coleoptile</td>
<td>5</td>
<td>48</td>
<td>2</td>
<td></td>
<td>Albaun and Eichel (1943)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>96</td>
<td>Stim 15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato stems</td>
<td>5</td>
<td>—</td>
<td>10</td>
<td></td>
<td>Eberts et al. (1951)</td>
</tr>
<tr>
<td>Corn roots</td>
<td>0.2</td>
<td>—</td>
<td>Stim</td>
<td></td>
<td>Biber and Farbman (1951)</td>
</tr>
<tr>
<td>Wheat roots</td>
<td>0.1</td>
<td>2</td>
<td>Stim 96</td>
<td></td>
<td>Lundegårdh (1944)</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>Stim 140</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2</td>
<td>Stim 225</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frog muscle</td>
<td>0.1</td>
<td>4*</td>
<td>10</td>
<td>0.95</td>
<td>0.89</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>4</td>
<td>22</td>
<td>0.95</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>4</td>
<td>31</td>
<td>0.94</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>4</td>
<td>37</td>
<td>0.94</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4</td>
<td>45</td>
<td>0.94</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>4</td>
<td>42</td>
<td>0.94</td>
<td>0.72</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>4</td>
<td>39</td>
<td>0.94</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>4</td>
<td>40</td>
<td>0.94</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>4</td>
<td>56</td>
<td>0.94</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>4</td>
<td>52</td>
<td>0.94</td>
<td>0.73</td>
</tr>
<tr>
<td>Tissue</td>
<td>( m )</td>
<td>( c )</td>
<td>( c^2 )</td>
<td>( s )</td>
<td>( s^2 )</td>
</tr>
<tr>
<td>------------------------------</td>
<td>--------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>Frog muscle mince</td>
<td>50</td>
<td>3(^a)</td>
<td>12</td>
<td>0.89</td>
<td>0.82</td>
</tr>
<tr>
<td>Rabbit muscle mince</td>
<td>50</td>
<td>3</td>
<td>60</td>
<td>1.00</td>
<td>1.28</td>
</tr>
<tr>
<td>Chicken muscle mince</td>
<td>50</td>
<td>3</td>
<td>49</td>
<td>1.12</td>
<td>1.29</td>
</tr>
<tr>
<td>Pigeon muscle mince</td>
<td>50</td>
<td>3</td>
<td>48</td>
<td>1.00</td>
<td>1.01</td>
</tr>
<tr>
<td>Mouse muscle mince</td>
<td>50</td>
<td>3</td>
<td>46</td>
<td>1.05</td>
<td>1.10</td>
</tr>
<tr>
<td>Human muscle mince</td>
<td>50</td>
<td>3</td>
<td>17</td>
<td>0.99</td>
<td>0.93</td>
</tr>
<tr>
<td>Rabbit liver mince</td>
<td>50</td>
<td>3</td>
<td>12</td>
<td>0.78</td>
<td>0.75</td>
</tr>
<tr>
<td>Rabbit kidney mince</td>
<td>50</td>
<td>3</td>
<td>44</td>
<td>0.84</td>
<td>0.88</td>
</tr>
<tr>
<td>Pigeon heart mince</td>
<td>50</td>
<td>3</td>
<td>37</td>
<td>0.88</td>
<td>1.01</td>
</tr>
<tr>
<td>Bull spermatozoa</td>
<td>1</td>
<td>1</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>51</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.3</td>
<td>1</td>
<td>68</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>1</td>
<td>91</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea pig brain slices</td>
<td>20</td>
<td>2</td>
<td>77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea pig kidney slices</td>
<td>20</td>
<td>2</td>
<td>43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guinea pig heart slices</td>
<td>20</td>
<td>2</td>
<td>38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat testis slices</td>
<td>20</td>
<td>2</td>
<td>37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat spleen slices</td>
<td>20</td>
<td>2</td>
<td>56</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat Jensen sarcoma slices</td>
<td>20</td>
<td>2</td>
<td>90</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( a \) Muscle incubated with maleate for 30 min and respiration measured over 4 hr.

\( b \) Tissue minces incubated with maleate for 10 min and respiration measured over 3 hr.
the stimulation of aerobic glycolysis is almost abolished. If pyruvate were merely acting as a hydrogen acceptor from 3-phosphoglyceraldehyde, more lactate should be formed instead of less. Furthermore, Lardy and Phillips (1943 b) found that maleate does not reduce spermatozoa ATP, although motility is lost; ATP-forming reactions occur but the utilization of ATP is depressed. One may note here that the possible effects of maleate of ATPases have not been studied.

A clue to the mechanism of maleate action may be found in the unexpected effects of glutamate (Fig. 2-2). Although respiration in the presence of glutamate is readily depressed to low levels, there is a rapid and marked acceleration of aerobic glycolysis, which, however, is temporary. The rate of aerobic glycolysis actually reaches the anaerobic level in the presence of glutamate. It may be noted that α-ketoglutarate does not alter the response of aerobic glycolysis to maleate. This problem undoubtedly involves the regulation of pyruvate metabolism by NH$_4^+$ levels and transaminations. NH$_4^+$ is known to augment aerobic glycolysis in brain. Weil-Malherbe suggested that maleate may inhibit the utilization of NH$_4^+$ and hence lead to its accumulation, but analyses of the brain for NH$_4^+$ did not confirm this. It was shown later that maleate does not affect NH$_4^+$ formation in brain slices suspended in glucose medium (Weil-Malherbe and Green, 1955 a). NH$_4^+$ and glutamate may increase aerobic glycolysis by diverting cycle intermediates to reduce the supply of oxalacetate, less pyruvate entering the cycle to be oxidized. Glutamate might do this primarily through transamination with oxalacetate. However, we have seen that the aspartate:α-ketoglutarate transaminase is reasonably sensitive to maleate (Table 2-2). This may account for the early but temporary peak in aerobic glycolysis when maleate and glutamate are present, maleate penetrating into the slices and inhibiting rather slowly. The question whether the stimulation of aerobic glycolysis by maleate alone is related in any way to NH$_4^+$ metabolism or transamination remains to be answered. Since glutamate alone augments aerobic glycolysis and pyruvate alone depresses aerobic glycolysis in brain, the effects of these substances on the maleate response may be basically unrelated to the mechanism of maleate action. Data on the brain levels of keto acids and amino acids during treatment with maleate would help to solve this problem. We shall have occasion later to discuss the effects of maleate on amino acid metabolism and transamination in the whole animal (page 318).

**EFFECTS ON VARIOUS METABOLIC PATHWAYS**

Maleate has been shown to affect several important metabolic processes in microorganisms and tissues. In this section we shall discuss some of these in anticipation of the time when these observations may possibly be
correlated and a general picture of maleate action emerge, so that one can better understand the effects of maleate on growth and certain tissue functions in the whole animal.

**Amino Acid Synthesis in Liver and Kidney**

The synthesis of amino acids by transamination can under certain circumstances deplete the cycle, and this is probably one of the important regulatory mechanisms involved in the over-all response to maleate. If \( \alpha \)-ketoglutarate is incubated with rat liver mitochondria under the conditions used by Rogulski et al. (1962), 40% is oxidatively decarboxylated. When \( \text{NH}_4^+ \) is added, 90% of the \( \alpha \)-ketoglutarate is utilized and amino acids are formed; \( \text{NH}_4^+ \) in liver shifts the metabolism of \( \alpha \)-ketoglutarate toward amino acid synthesis. Addition of \( \text{NH}_4^+ \) to kidney mitochondria in the presence of \( \alpha \)-ketoglutarate, on the other hand, has much less effect and relatively little amino acid is formed. This system is more sensitive to maleate in the kidney than in the liver (Table 2-4) and similar results.

**Table 2-4**

**Effect of Maleate on \( \alpha \)-Ketoglutarate Metabolism in Liver and Kidney Mitochondria**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Maleate (mM)</th>
<th>% Inhibition of:</th>
<th></th>
<th>( \alpha )-Ketoglutarate removed/ amino acids formed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( \alpha )-Ketoglutarate removed</td>
<td>Amino acids formed</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>12</td>
<td>16</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>38</td>
<td>41</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>56</td>
<td>66</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>68</td>
<td>80</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>78</td>
<td>86</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>86</td>
<td>90</td>
<td>2.4</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>90</td>
<td>97</td>
<td>5.6</td>
</tr>
<tr>
<td>Kidney</td>
<td>0</td>
<td>—</td>
<td>—</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>7</td>
<td>0</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>25</td>
<td>22</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
<td>61</td>
<td>59</td>
<td>4.8</td>
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<td>77</td>
<td>76</td>
<td>4.8</td>
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<tr>
<td></td>
<td>3</td>
<td>87</td>
<td>92</td>
<td>9.0</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>90</td>
<td>98</td>
<td>(30.5)(^b)</td>
</tr>
</tbody>
</table>

\(^a\) Incubation at pH 7.2 and 38° for 1 hr, the initial concentration of \( \alpha \)-ketoglutarate being 20 mM and \( \text{NH}_4^+ \) 20 mM. (From Rogulski et al., 1962.)

\(^b\) Value is approximate because so little amino acid formed.
were obtained \textit{in vivo} (see page 319). The reactions in liver mitochondria may be written as:

\[ \alpha\text{-Ketoglutarate} + \text{NAD}^+ \rightarrow \text{succinate} + \text{CO}_2 + \text{NADH} + \text{H}^+ \]

\[ \alpha\text{-Ketoglutarate} + \text{NH}_4^+ + \text{NADH} \rightarrow \text{glutamate} + \text{NAD}^+ \]

\[ 2 \alpha\text{-Ketoglutarate} + \text{NH}_4^+ \rightarrow \text{succinate} + \text{glutamate} + \text{CO}_2 + \text{H}^+ \]

the reductive amination of \( \alpha \)-ketoglutarate utilizing the NADH formed in the oxidation of the remaining \( \alpha \)-ketoglutarate, since there are approximately 2 moles of \( \alpha \)-ketoglutarate removed for each mole of amino acid formed. In the kidney around 5 moles of \( \alpha \)-ketoglutarate disappear for each mole of amino acid formed. It is difficult to decide which reaction is inhibited by maleate, but the relative constancy of the \( \alpha \)-ketoglutarate/amino acid ratio led Rogulski \textit{et al.} to assume that the primary effect is on the oxidation of \( \alpha \)-ketoglutarate. Certainly the \( \alpha \)-ketoglutarate oxidation in kidney homogenates is quite sensitive to maleate, whereas that in liver is not (Angielski and Rogulski, 1962). The moderate rise in the ratio could be explained by a less potent inhibition of the amination reaction, but also by less efficiency in the utilization of NADH when little is formed. In any event, these results indicate a remarkable difference between liver and kidney with respect to the response to maleate, and this has important bearing on the results obtained in whole animals.

**Organic Acid Metabolism in Tobacco Leaves**

Perhaps the most thorough and interesting study of the complex effects of maleate on metabolism is that of Vickery and Palmer (1956 a), using tobacco leaves cultured with maleate, fumarate, or succinate at 200 mM and pH 5 for 48 hr. It was known that fumarate is well utilized by these leaves and that maleate often increases the respiration of plant tissues; thus an investigation was made into the question of whether maleate is utilized and whether it exhibits inhibitory effects on metabolism. The effects are summarized in Table 2-5. It is evident that maleate is metabolized to some extent, but this will be discussed in a later section (page 314), and here we shall consider only the evidence for an inhibitory action on certain phases of the leaf metabolism. It is first necessary to calculate the final concentration of maleate in the leaf water: the leaves initially weighed 1000 g and lost 489 g during 48 hr, so that final weight was 511 g; total solids were 122 g, so that 389 g of water were present; the leaves contained 239 meq of maleate, so the concentration is 310 mM. If the uptake was linear, as it is for fumarate, the concentration must have been over 100 mM for 24 hr or more, so that there would appear to be both ample time and concentration for inhibition to be exerted.
Table 2-5
EFFECTS ON VARIOUS METABOLIC PATHWAYS

<table>
<thead>
<tr>
<th></th>
<th>Initial value</th>
<th>Water control</th>
<th>Maleate</th>
<th>Fumarate</th>
<th>Succinate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh weight (g)</td>
<td>1000</td>
<td>+167</td>
<td>-489</td>
<td>+77</td>
<td>+129</td>
</tr>
<tr>
<td>Transpiration (ml)</td>
<td>—</td>
<td>—</td>
<td>1210</td>
<td>348</td>
<td>360</td>
</tr>
<tr>
<td>Organic solids (g)</td>
<td>93.1</td>
<td>- 5.5</td>
<td>+ 8.4</td>
<td>+ 1.6</td>
<td>+ 1.8</td>
</tr>
<tr>
<td>Uptake of acid (meq)</td>
<td>—</td>
<td>—</td>
<td>+289</td>
<td>+171</td>
<td>+226</td>
</tr>
<tr>
<td>Total organic acids (meq)</td>
<td>283</td>
<td>- 8.1</td>
<td>+258</td>
<td>+165</td>
<td>+139</td>
</tr>
<tr>
<td>Malate (meq)</td>
<td>198</td>
<td>- 62.6</td>
<td>- 18.8</td>
<td>- 17.0</td>
<td>+ 38.3</td>
</tr>
<tr>
<td>Citrate (meq)</td>
<td>22.7</td>
<td>+ 50.1</td>
<td>+ 21.7</td>
<td>+ 96.8</td>
<td>+ 74.1</td>
</tr>
<tr>
<td>Oxalate (meq)</td>
<td>32.8</td>
<td>+ 3.9</td>
<td>+ 1.1</td>
<td>+ 3.9</td>
<td>+ 0.2</td>
</tr>
<tr>
<td>Fumarate, maleate, or succinate (meq)</td>
<td>1</td>
<td>—</td>
<td>+239</td>
<td>+86.4</td>
<td>+ 18</td>
</tr>
<tr>
<td>Unknown acid A (meq)</td>
<td>18</td>
<td>+ 0.3</td>
<td>+ 9.5</td>
<td>- 7.2</td>
<td>+ 2.5</td>
</tr>
<tr>
<td>Protein nitrogen (g)</td>
<td>3.07</td>
<td>- 0.24</td>
<td>- 0.16</td>
<td>- 0.44</td>
<td>- 0.38</td>
</tr>
<tr>
<td>Starch (g)</td>
<td>2.07</td>
<td>- 1.8</td>
<td>- 1.5</td>
<td>- 1.9</td>
<td>- 2.0</td>
</tr>
<tr>
<td>Acquired acid metabolized (meq)</td>
<td>—</td>
<td>—</td>
<td>51</td>
<td>84</td>
<td>209</td>
</tr>
<tr>
<td>Acquired acid metabolized (% of that taken up)</td>
<td>—</td>
<td>—</td>
<td>18</td>
<td>49</td>
<td>92</td>
</tr>
<tr>
<td>Acquired acid converted into different acid (meq)</td>
<td>—</td>
<td>—</td>
<td>19</td>
<td>78</td>
<td>122</td>
</tr>
</tbody>
</table>

* Leaves of *Nicotiana tabacum* cultured for 48 hr at pH 5 and 24°. Maleate, fumarate, and succinate were 200 mM. (From Vickery and Palmer, 1956 a.)
There are three changes which may be taken as evidence for enzyme inhibition. (1) The loss of protein nitrogen is stimulated by fumarate and succinate, but is inhibited 33% by maleate. It was believed that this is due to inactivation of proteolytic enzymes. (2) The formation of citrate is again stimulated by fumarate and succinate, but is depressed 57% by maleate. (3) Malate disappeared in the controls, presumably by being transformed mainly to citrate, and fumarate spared this malate by being itself metabolized through malate to citrate, whereas maleate reduced the malate that disappeared. This can hardly be explained on the same basis as fumarate, since so little of the maleate taken up is converted into malate or citrate. Thus an inhibition of malate utilization can be inferred. It should be noted that the leaves cultured in water, fumarate, or succinate maintained their turgidity and even increased in weight, whereas those cultured in maleate became flaccid and in 48 hr had lost almost half their weight. The effects on organic acid metabolism by maleate can be satisfactorily explained by a primary effect on the oxidation of pyruvate and α-ketoglutarate, but it is possible that unrecognized inhibitions may be more important.

The effects of maleate at pH 6 are not basically different from those presented above for pH 5. It is surprising that about 12% more acid is taken up at pH 6. This is opposite to that found for succinate, malate, fumarate, and citrate, and to what might be expected on the basis of the penetration of the less ionized forms. The final maleate concentration in the leaves at pH 6 is actually somewhat less than at pH 5, but this is due mainly to a much greater metabolism of maleate at pH 6.

Metabolism of Trilaurin in Rat Liver Slices

Trilaurin labeled in the carboxyl groups incubated with liver slices gives rise to C14O2. The effects of fumarate and maleate are quite different (see accompanying tabulation). Although maleate does not affect the total

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Fumarate (5 mM)</th>
<th>Maleate (5 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total respired CO₂</td>
<td>100</td>
<td>152</td>
<td>101</td>
</tr>
<tr>
<td>Total respired C¹⁴O₂</td>
<td>100</td>
<td>126</td>
<td>69</td>
</tr>
<tr>
<td>Specific activity of CO₂</td>
<td>100</td>
<td>83</td>
<td>68</td>
</tr>
</tbody>
</table>

CO₂ produced, it depresses the appearance of C¹⁴O₂. The effects are similar to those produced by malonate, but not as marked (Geyer et al., 1950 a). The action of maleate here could be entirely on the cycle and not necessarily on the specific reactions involving oxidation of fatty acids. Nothing is known of the effects of maleate on the enzymes of the fatty acid helix.
Porphyrin Synthesis

Fumarate at 10 mM augments porphyrin synthesis in chicken erythrocytes incubated with 70 mM glycine, but 10 mM maleate inhibits 88% (Granick, 1958). Since an active cycle is required for the formation of succinyl-CoA to condense with glycine in the initial step of porphyrin synthesis, one might immediately surmise that the inhibition is possibly on α-ketoglutarate oxidase. The synthesis of δ-aminolevulinate by guinea pig liver mitochondria incubated with glycine and citrate is inhibited 44% by 10 mM maleate (Granick and Urata, 1963), so that a definite effect on the early stages of synthesis is clear. However, the exact site for the inhibition is not clear. Granick and Urata suggested cis-aconitase as the sensitive enzyme, but I know of no direct evidence that this enzyme is affected by maleate (see Table 2-2). Inasmuch as maleate at 10 mM would certainly inhibit α-ketoglutarate oxidase to some extent, even in liver, during a 2 hr incubation, this might also be considered as the site of action. The δ-aminolevulinate synthetase itself may well be susceptible to maleate but has not been tested directly.

Cysteine Metabolism in Entamoeba and E. Coli

The formation of H₂S and CO₂ from glucose and cysteine under anaerobic conditions by Entamoeba histolytica, probably an important pathway in this organism, involves the formation of a labile sulfur compound (possibly β-thiopyruvate) by reaction of cysteine with pyruvate, the release of H₂S from this intermediate, and the reduction of the cystine formed by 3-phosphoglyceraldehyde to restore the cysteine. The formation of H₂S is inhibited 46% and of CO₂ 14% by 20 mM maleate (Kun et al., 1955). Although a number of enzymes participate in this system, possibly maleate reacts directly with cysteine or β-thiopyruvate, the production of H₂S being affected more than the release of CO₂. In E. coli, cysteine is metabolized to H₂S and ammonia, and Tamiya (1954) postulated an oxidative deamination involving β-thiopyruvate as an intermediate. Maleate at 5 mM has no effect on this pathway. Although the concentration here is less than in the work with Entamoeba, the results make it less likely that maleate reacts with cysteine or β-thiopyruvate with sufficient rapidity to account for the former inhibition.

METABOLISM OF MALEATE

The possible utilization of maleate has always been a complicating factor in studying metabolic inhibition by this substance in tissues. Much work has shown that fumarate can be readily utilized, whereas maleate generally is not, although definite metabolism of maleate has been demonstrated in
certain instances. It should be clear that slight to moderate stimulation of respiration by maleate does not constitute sufficient evidence for its metabolism. In studying the metabolism of maleate, one must be particularly careful to use a preparation free from fumarate, and this factor makes some of the early work difficult to interpret because the purity of the maleate used is unknown.

Organisms and tissue preparations may be divided into three categories with respect to the utilization of maleate.

(A) Maleate not utilized

Bacteria: *Bacillus subtilis, E. coli, Proteus, Pseudomonas, Salmonella, Shigella* (Nicolle and Joyeux, 1950), and 14 strains of *Pseudomonas* (Shilo and Stanier, 1957)

Fungi: *Aspergillus* and *Penicillium* sp. (Berk et al., 1957)

Yeast: *Saccharomyces* (Jung and Müller, 1922)

Animal tissues: frog muscle (Dakin, 1922) and beef testis and kidney (Hallman, 1938, 1940)

(B) Maleate definitely metabolized

Bacteria: *Klebsiella pneumoniae* (Nicolle and Joyeux, 1950) and *Pseudomonas* strain Maleic 5 (Shilo and Stanier, 1957)

Plants: wheat roots (Lundegårdh, 1944) and tobacco leaves (Vickery and Palmer, 1956 a)

Animals: dogs (Sacks, 1958) and rats (Taggart et al., 1962)

(C) Maleate possibly metabolized (evidence not clear-cut) or slightly metabolized

Bacteria: *Azotobacter* (< 4% metabolized) (Lineweaver, 1933) and *Rhizobium* strains (Burris and Wilson, 1939)

Plants: bean and tomato plants (Greulach, 1953)

Animals: rats (Krusius, 1940), rabbits (Krebs *et al.*, 1938), and dogs (Orten and Smith, 1937) — evidence in all these from increased citrate excretion

There are, of course, a number of instances in which maleate has been used and no evidence for its metabolism has been noted, although this was not tested directly. The results are equivocal in some of the early work and the conclusions reached are now subject to doubt. Ohta (1912) reported that dog livers perfused with maleate formed acetoacetate, and believed that maleate was converted to acetoacetate, but possibly this is the result of a disturbance in the metabolism brought about by maleate. Thunberg (1920) found that maleate decolorized methylene blue in the presence of frog muscle mince and discussed the possibility that it was oxidized to acetylenedicarboxylate. Laki (1935) thought that maleate can be reduced like fumarate in extracts from muscle, but this has not been confirmed. Fabisch (1931) reported the esterification of maleate in the presence of
acetone powders of pig tissues and various alcohols, but it is unlikely that this could be important in the intact tissues.

The most obvious reactions by which maleate could be metabolized are: (1) isomerization to fumarate, (2) hydration to malate, (3) reduction to succinate, (4) oxidation to meso-tartrate, and (5) decarboxylation to acrylate. Enzymes for only the first two reactions have been reported, but the other pathways cannot be neglected, although they are less likely to occur. A cis-trans isomerase that converts maleate to fumarate was isolated from *Pseudomonas* sp. by Otsuka (1961). This enzyme is fairly active and sufficient reaction occurs in several minutes to make readings. An enzyme converting maleate to malate was obtained from ground corn by Sacks and Jensen (1951) and called malease. The activity seems to be quite low, since when maleate was 1 mM initially, 2% reacted in 1.5 hr, 6% in 7 hr, 18% in 12 hr, and 95% in 24 hr. Unfortunately, the distribution of these enzymes is not known and hence their importance generally in the metabolism of maleate cannot be assessed.

Let us turn to studies purporting to demonstrate maleate utilization in whole animals in order to determine which pathways may be operative. Orten and Smith (1937) injected the sodium salts of different acids intravenously at a dose of 4.35 millimoles/kg in dogs, and determined the urinary citrate over a 24-hr period. The results are given in Table II-1-22 along with data from higher doses in rats provided by Simola and Kosunen (1938) and Krusius (1940). There is no doubt that maleate can markedly increase the excretion of citrate, usually more so than most of the other organic acids. The question is whether maleate serves as a precursor for citrate, presumably thorough fumarate or malate, or produces an accumulation of citrate as a result of some inhibition in the cycle. Krebs et al. (1938) showed that in rabbits not only urinary citrate is elevated by maleate, but also α-ketoglutarate and succinate (7.5 and 2.5 times, respectively). They pointed out that maleate acts similarly to malonate, but that metabolism of maleate can not be excluded. It seems to me that these results cannot be taken as evidence for maleate metabolism, but are more important for demonstrating inhibitory effects on the cycle *in vivo*. If maleate goes to citrate through fumarate or malate, it is odd that maleate is usually somewhat more effective than fumarate or malate in elevating the urinary citrate.

A clear demonstration of maleate metabolism in dogs and man was made by Sacks (1958), who administered maleate-2-C\(^{14}\) and determined the blood levels of C\(^{14}\)O\(_2\) at intervals. In man the specific activity of the CO\(_2\) reaches a maximum in around 60-70 min, so the metabolism occurs fairly rapidly, although not nearly as rapidly as the metabolism of fumarate. No data on the extent of the conversion were given. Since it was thought that the first step might be the conversion of maleate to fumarate, labeled fumarate in
the blood was determined but little activity was found. Thus there was no evidence for the pathway

\[
\text{Maleate} \rightarrow \text{fumarate} \rightarrow \text{CO}_2
\]

but it is possible that within the tissues the fumarate is so rapidly metabolized that little escapes into the blood.

A very interesting pathway for maleate metabolism has recently been established by Taggart et al. (1962). When maleate-\(^1\)\(^4\)C\(^{14}\) or maleate-\(^2\)\(^3\)C\(^{14}\) is injected intraperitoneally in rats, C\(^{14}\)O\(_2\) appears faster with the former; at 4 hr the C\(^{14}\)O\(_2\) recovered is 40\% and 25\%, respectively. The radioactivity in the urine is mainly maleate. If maleate-\(^1\)\(^4\)C\(^{14}\) is incubated with kidney preparations, C\(^{14}\)O\(_2\) is released; liver preparations are essentially inactive. Neither kidney mitochondria nor supernate are able alone to convert maleate to CO\(_2\), but together the reaction proceeds readily (see accompanying tabulation). Incubation of maleate-\(^1\)\(^4\)C\(^{14}\) with the supernate

<table>
<thead>
<tr>
<th>Preparation</th>
<th>% C(^{14})O(_2) recovered in 1 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney slices</td>
<td>19</td>
</tr>
<tr>
<td>Kidney homogenate</td>
<td>44</td>
</tr>
<tr>
<td>Kidney mitochondria (washed)</td>
<td>4</td>
</tr>
<tr>
<td>+ 0.2 ml supernate</td>
<td>12</td>
</tr>
<tr>
<td>+ 0.5 ml supernate</td>
<td>26</td>
</tr>
<tr>
<td>+ 1 ml supernate</td>
<td>35</td>
</tr>
</tbody>
</table>

yields 60–90\% of a product shown chromatographically to be D-malate. The D-malate is readily converted to CO\(_2\) by the kidney mitochondria after entering the cycle by means of a NAD-independent D-2-hydroxyacid dehydrogenase. The supernate enzyme is thus a maleate hydratase, or malEase, but presumably different from that isolated from corn. Possibly the renal metabolism of maleate can give rise to some urinary citrate, but it seems unlikely that this is the major factor involved.

Maleate is utilized in tobacco leaves to a lesser extent than either fumarate or succinate, 18\% of the maleate taken up by the tissue being metabolized (Vickery and Palmer, 1956 a). Apparently about half of the metabolized maleate goes to CO\(_2\) and the rest to organic acids. From the data in Table 2-5 it can be calculated that an appreciable amount of maleate is converted to some acid or acids other than malate or citrate, whereas fumarate is ultimately all converted to citrate. The metabolism of maleate here thus seems to be more complex than a simple isomerization to fumarate and its subsequent metabolism.
EFFECTS ON TISSUE FUNCTION

Very little investigation of the effects of maleate on cellular activity has been done, the exception being the work on renal function to be discussed in the next section. Surprisingly sparse are the reports on the response of muscle to maleate. Kuschinsky and Lüllmann (1954) observed that maleate at 1.7–8.6 mM causes a prolonged contracture of the diaphragm as do various SH reagents, and Hasselbach (1953) stated that 50 mM maleate inhibits the relaxation of glycerinated muscle fibers. Even less has been done on the heart and we find only that maleate and fumarate both depress the isolated frog heart, the former more strongly and less reversibly (Lipschitz and Hertwig, 1921). Inasmuch as I know of no other published study of the effects of maleate on cardiac muscle, some preliminary experiments may be mentioned (Webb and Hollander, 1958). Maleate at 5 mM causes a 20% depression of the contractility of rat atria, and at 15 mM the depression is 35% within 30 min, while both the resting and action potential magnitudes are reduced moderately without significant alteration of the depolarization and repolarization rates. The lack of a shortening effect on the action potential duration was unexpected, since this is a common property of metabolic inhibitors, and it probably indicates that not enough maleate penetrates into the myocardial cells to affect the cycle. The rate at which the depression develops is very rapid, so as to lead one to suspect an action on the cell membranes. Rjabinovskaja (1939) studied the effects of maleate on a neuromuscular preparation from frogs, since he believed that maleate is a glycolytic inhibitor and, hence, might interfere with the synthesis of acetylcholine. When maleate is applied at 2.5 mM neuromuscular block definitely occurs before a depression of the muscle. The block is established rather rapidly and it is doubtful if it is in any way related to glycolytic inhibition. One recalls that work with the mercurials may indicate SH groups on the acetylcholine receptors (Turpaev, 1955; Nistratova and Turpaev, 1959), so the effects of maleate on the response of the muscle to acetylcholine should be examined. Essentially nothing is known about the actions of maleate on nerves, and it is likely that penetration into axons must be limited. Yet Greengard and Straub (1962) report that 0.15 mM maleate produces a small decrease in the posttetanic hyperpolarization of sympathetic C fibers. From the results on atria mentioned above, the neuromuscular block, and this effect on nerve fibers at a low concentration, it seems that a study of maleate with respect to its actions directly on cell membranes might be interesting. The observation of Lundegårdh (1944) that maleate causes a rapid fall in the membrane potential of wheat root cells is also evidence for a membrane effect.

Spermatozoal motility is depressed by maleate but not by fumarate. Lipschitz and Hertwig (1921) found that frog spermatozoa are unaffected
after 37 min in 43 mM fumarate, whereas in the same concentration of maleate there is slowing in 3 min, marked depression in 16 min, and paralysis in 37 min. Maleate at 21.5 mM also paralyzed but it required 75 min. Bull spermatozoa are even more sensitive, their motility being fairly rapidly and irreversibly abolished by 5–10 mM maleate (Lardy and Phillips, 1943 a, b). We have mentioned that this is not associated with a fall in the ATP level and is accompanied by an acceleration of aerobic glycolysis. It was postulated that maleate inactivates spermatozoa by preventing the utilization of ATP.

The inflammatory and vesicant effects of various SH reagents point to an important role of SH enzymes in the skin, so it is interesting to inquire if maleate exerts an action on this tissue. Industrial exposure to maleic acid and maleic anhydride fumes has led to severe conjunctivitis, vesicular dermatitis, and upper respiratory tract irritation. Winter and Tullius (1950) for this reason investigated the effects of these substances on the eyes of rabbits. When the conjunctival sac is filled with a solution of maleic acid at 86 mM (1%) for 2 min and then drained, corneal cloudiness, conjunctival hyperemia, and edema of the nictitating membrane occur within a few minutes, and this lasts for several hours, the eyes being normal after 1 day. A 430 mM (5%) solution causes a more intense irritation which involves the iris as well as the cornea, and the effects do not disappear until the sixth or seventh day. Maleic anhydride produces similar effects. The ocular reflexes are unaffected and no systemic reactions occur. Since they apparently did not neutralize the maleic acid, it is difficult to determine how much of the action is due to maleate and how much to a nonspecific acid injury. One might well expect acetic acid, or other organic acids, at these concentrations to be quite irritant.

**EFFECTS ON WHOLE ANIMALS**

Minimal toxic and lethal doses for maleate have never been determined accurately. Doses in the range 200–400 mg/kg* (1.7–4.3 millimoles/kg) subcutaneously or intraperitoneally to rats produce marked changes in the blood and urinary keto acids, glucose, amino acids, and pH (Krebs et al., 1938; Harrison and Harrison, 1954; El Hawary, 1955; Angielski et al., 1959). However, these doses do not usually produce significant toxic reactions. The lethal dose in dogs is around 190–250 mg/kg (1.6–2.2 millimoles/kg) given intravenously (Orten and Smith, 1937; Hermann et al., 1938). Fumarate is much less toxic, Foderá (1894) reporting that even 1940 mg/kg (17 millimoles/kg) intravenously to dogs produces only minor reac-

* All doses are given in terms of maleic acid; in most cases the solutions were neutralized before injection.
tions. Fitzhugh and Nelson (1947) fed diets containing various organic acids to rats and found that 0.1–1.5% fumaric acid is without effect on the weight gain. On the other hand, maleic acid at 0.5% minimally inhibits growth (4%), at 1% definitely suppresses growth (20%), and at 1.5% not only markedly reduces growth (41%) but increases the mortality rate so that all the rats die within 2 years. Autopsy examinations showed large and irregularly shaped epithelial cells in the renal tubules, some atrophy of the liver and testes, and less focal calcification of the large arteries. It is possible that the tissue atrophies were due in part to the general state of inanition. No other tissues showed demonstrable changes.

The effects of maleate on urinary citrate and keto acids have been discussed (Orten and Smith, 1937; Krebs et al., 1938; Krusius, 1940). The studies of El Hawary (1955) on the effects of SH reagents on the levels of keto acids, ketone bodies, and glucose in the blood of rats are pertinent to the problem of the metabolic disturbances produced by maleate. Administration of maleate at 420 mg/kg (3.6 millimoles/kg) intraperitoneally produces the changes given in the accompanying tabulation at 90 min.

<table>
<thead>
<tr>
<th>Substances</th>
<th>Ratio of levels in treated animals to levels in control animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>1.5</td>
</tr>
<tr>
<td>Keto acids</td>
<td>3.9</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>2.7</td>
</tr>
<tr>
<td>α-Ketoglutarate</td>
<td>9.3</td>
</tr>
<tr>
<td>Acetone and acetoacetate</td>
<td>1.8</td>
</tr>
</tbody>
</table>

Since maleate inhibits the oxidation of pyruvate and α-ketoglutarate, the elevations of the blood levels of these substances might be expected. The greater rise of α-ketoglutarate relative to pyruvate was thought to be due in part to the metabolism of maleate through fumarate, inasmuch as fumarate has been shown to elevate α-ketoglutarate in tissues. If pyruvate is injected 90 min after the administration of maleate, the blood levels of pyruvate actually increase gradually over 30 min, whereas in normal animals the pyruvate level falls rapidly (see accompanying tabulation).

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Blood pyruvate (mg%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Maleate</td>
</tr>
<tr>
<td>5</td>
<td>6.25</td>
</tr>
<tr>
<td>15</td>
<td>2.54</td>
</tr>
<tr>
<td>30</td>
<td>1.80</td>
</tr>
</tbody>
</table>
This is the best evidence we have that maleate inhibits the utilization of pyruvate in vivo.

**Effect on Renal Acidification Mechanisms**

Orten and Smith (1937) noted that maleate increases the urinary pH in dogs when administered as the sodium salt, an action it shares with the sodium salts of acetate, succinate, malate, and other organic acid anions. This would be expected, but Hermann et al. (1938) found that even malic acid, injected at pH 1.5–1.7, causes a rise in blood pH, whereas fumaric acid does not in a comparable dose, although this may not depend on renal action. The most interesting results were obtained by Berliner et al., (1950), who administered rather small doses (40 mg/kg) intravenously to dogs in severe acidosis (plasma pH 7.0, CO₂ concentration 9.5 mM) and found a prompt rise in the urinary pH, significant after 15 min and persisting for 3–4 hr. In some cases the urinary pH rose to that of the plasma, and in one experiment from 6.0 to 7.38, while the titratable acidity fell from 203 to 0. Transport of p-aminohippurate was depressed slightly and the resorption of glucose was reduced substantially. The most striking effect of maleate is impairment of the mechanism for acidification of the urine and bicarbonate resorption. Most of the cation loss is due to Na⁺ excretion, but K⁺ excretion rises moderately, no effect being noted on Cl⁻ excretion; there is probably very little direct effect on the transport of Na⁺, K⁺, or Cl⁻. This is substantiated by the results of Mudge (1951) with rabbit kidney slices, in which maleate at 10 mM not only does not interfere with the restoration of K⁺ in depleted slices, but actually seems to accelerate the uptake of K⁺ and extrusion of Na⁺. A direct effect of maleate on the tubular cells to alter their function was demonstrated by Berliner et al. by administering glucose with the maleate. Maleate alone produces a completely reversible disturbance of renal function, but with glucose is more toxic, some of the dogs dying within 2–3 days while others presented an elevation of blood urea N and a persistent acidosis, post-mortem showing a diffuse necrosis of the convoluted tubules. Maleate, thus, exerts a rather unique effect on the kidneys, the mechanism of which is still unknown.

**Aminoaciduria and Glucosuria**

When rats on a rachitogenic diet (high Ca⁺⁺ and low phosphate) are given maleate intraperitoneally, the urinary phosphate excretion rises markedly (around 35-fold on the first day), as do the excretions of glucose (28-fold) and amino acids (7.5-fold) (Harrison and Harrison, 1954). The plasma levels of phosphate and glucose are not elevated (in fact, blood glucose falls somewhat), so the site of action appears to be on the kidney.
It is interesting that this triad of glucosuria, phosphaturia, and aminoaciduria is characteristic of a congenital metabolic defect in man known as the Fanconi syndrome, and in this disease it is common to find rickets resistant to vitamin D. It was felt that an elucidation of the mechanism by which maleate produces these renal effects might give some insight into the metabolic derangement responsible for this disease.

The aminoaciduria and associated urinary changes produced by maleate have been studied in detail by Angielski and his colleagues in Poland. At a dosage of 300–400 mg/kg, maleate increases the urinary amino acid excretion 5- to 10-fold in rats, the maximal effect occurring on the second or third day, recovery to normal levels taking place after a week (Angielski and Rogulski, 1959 a). The aminoaciduria occurs when the rats are on a diet containing milk or lactose, but not on a lactose-free diet. One week on the lactose diet is required to render the rats sensitive to maleate (Angielski et al., 1959). The degree of aminoaciduria also depends on the vitamin intake and tocopherol reduces it. There are actually two phases which may be distinguished. The initial phase of action occurs during the first 2 hr after administration and is characterized by increased urinary excretion of amino acids, keto acids, glucose, and ammonia, and by diuresis. The second phase lasts for several days and is characterized by aminoaciduria and an even greater diuresis (Angielski et al., 1960 c). The initial phase is independent of the diet, whereas the second phase depends on lactose in the diet. The relationship between maleate aminoaciduria and lactose has been intensively studied, but the mechanism of the dependence has not been elucidated. Handler (1947) stated that rats on a high galactose diet die in a few days and exhibit renal damage and various other changes similar to those produced by maleate. Rats on a galactose diet excrete high levels of the sugar; administration of maleate brings about a rapid and marked fall in galactose excretion (Angielski et al., 1961). On the other hand, rats on a milk diet respond by a rise in galactose excretion. Angielski and his co-workers attribute these effects of maleate to disturbances in galactose metabolism. The rate of conversion of galactose to CO₂ is believed to be controlled by the epimerization of UDP-galactose. Since this reaction depends on catalytic concentrations of NAD, and is strongly inhibited by NADH, anything that alters the NAD:NADH ratio may affect galactose metabolism. A rise in NADH would reduce galactose oxidation and presumably augment its urinary excretion. Possibly these factors are important in the effects of maleate but there is no direct evidence that maleate alters the NAD:NADH ratio.

The plasma amino acid level does not rise during maleate aminoaciduria so that the site of action of maleate must be renal (Angielski et al., 1960 b). The possibility that maleate exerts its effects by reacting with thiols was examined by determination of the SH groups in blood, liver, and kidneys
at 15 min, 3 hr, and 48 hr after administration (Rogulski, 1960). No changes in blood or liver SH groups occur at any time, but in the kidney there is considerable reduction in both protein and nonprotein SH groups within 15 min. In rats on a lactose diet this reduction amounts to as much as 80%. Protein SH is restored at 3 hr but nonprotein SH remains subnormal for much longer. These data also point to the kidney as the primary site of action of maleate, and Rogulski, compared maleate with the mercurials with respect to their actions on the kidney. It is rather unexpected that dimercaprol given intramuscularly is able to abolish the maleate amino-aciduria completely, while glutathione and cysteine are partially effective (Angielski and Rogulski, 1959 b), inasmuch as the rates of reaction of maleate with these thiols do not appear to be sufficiently rapid to account for the inactivation of the maleate in vivo.

A very interesting comparison of maleate with several other enzyme inhibitors was made by Angielski et al. (1960 a). Considerable diuresis, glucosuria, aminoaciduria, and albuminuria are produced by both maleate and the mercurials, but keto acid excretion is distinctly decreased by the mercurials in contrast to the ketoaciduria seen with maleate. Some of the results are summarized in Table 2-6, from which it is clear that iodoacetate does not share the actions of maleate, whereas malonate produces on the whole a similar urinary picture, although the aminoaciduria is not so severe. One might conclude that maleate may produce its renal effects by inhibition of the cycle, but probably this contributes only to a minor extent, and the primary action is on some system, possibly involving SH groups, responsible for tubular transport and amino acid metabolism. One recalls that the oxidation of α-ketoglutarate and the formation of amino acids in kidney mitochondria are potently inhibited by maleate at concentrations around 1 mM (Table 2-4) and that the dose for maleate aminoaciduria in rats would lead to an over-all concentration of maleate in the body water of around 3–4 mM (Niemiro, 1960). The hypothesis might be entertained that maleate effectively blocks keto acid oxidation, leading to a moderate rise in keto acid excretion, and that part of the keto acid accumulated is aminated to amino acids, which are excreted, this also leading to impairment of the acidification mechanisms in the kidneys. The ammonia required for the amination could come from other amino acids which the kidney normally deaminates and the final result would be an increased urinary amino acid level. On the other hand, the entrance of filtered amino acids into the tubular cells or their metabolism might be interfered with in some manner by maleate. It would be interesting to know exactly which amino acids are elevated in the urine during maleate aminoaciduria.
Table 2-6

Effects of Inhibitors on the Urinary Excretion Pattern in Rats

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>% Change in:</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st day</td>
<td>2nd day</td>
<td>3rd day</td>
<td>1st day</td>
<td>2nd day</td>
<td>3rd day</td>
</tr>
<tr>
<td>Maleate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+127</td>
<td>+164</td>
<td>+109</td>
</tr>
<tr>
<td>Mersalyl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+184</td>
<td>+167</td>
<td>+284</td>
</tr>
<tr>
<td>HgCl₂</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+22</td>
<td>+22</td>
<td>+211</td>
</tr>
<tr>
<td>Malonate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+150</td>
<td>+75</td>
<td>+87</td>
</tr>
<tr>
<td>Phlorizin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+100</td>
<td>+29</td>
<td>+43</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-31</td>
<td>-38</td>
<td>-38</td>
</tr>
</tbody>
</table>

a The inhibitors were given subcutaneously in the following doses (millimoles/kg): maleate 3.47, mersalyl 0.04, HgCl₂ 0.0075, malonate 10, phlorizin 1.06, and iodoacetate 0.108. (From Angielski et al., 1960 a.)
EFFECTS ON MITOSIS AND GROWTH

Many naphthoquinones are potent antimitotic agents and react readily with thiols. Friedmann et al. (1948 a) reasoned that since many of the chemical properties of the naphthoquinones relate to the nonbenzenoid part of the molecule, the antimitotic activity might also, and so they investigated maleate (which is 1,4-naphthoquinone with the benzene ring replaced by two hydroxylate groups), using chick fibroblast cultures. The shift in the phase distribution produced by 0.002 mM maleate (see accompanying tabulation) points to a metaphase-type inhibition. This is substantiated by the cytological changes observed: clumped metaphases, fragmentation of chromosomes, undivided telophases, and, in higher concentrations, exploded cells. There is a linear relationship between log(inhibition) and log(maleate), 50% inhibition being given by 0.0005 mM. The potency of maleate here is remarkably high and it is difficult to attribute the antimitotic action to an effect on enzymes or metabolism.

<table>
<thead>
<tr>
<th>% in prophase</th>
<th>Controls</th>
<th>Maleate</th>
</tr>
</thead>
<tbody>
<tr>
<td>% in metaphase</td>
<td>17</td>
<td>19</td>
</tr>
<tr>
<td>% in anaphase</td>
<td>31</td>
<td>40</td>
</tr>
<tr>
<td>% in telophase</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>% decrease in mitoses</td>
<td>47</td>
<td>36</td>
</tr>
</tbody>
</table>

Fumarate produces some abnormal cells but does not inhibit mitosis, while methylmaleate (citraconate) and methylfumarate (mesaconate) are completely inactive, indicating effective blocking of the double bond by the methyl group. Is reaction with SH groups involved in the antimitotic action of maleate? Friedmann et al. (1948 b) prepared and examined the addition compounds of maleate with glutathione, thiolacetate, and cysteine, and showed them to be antimitotic, although less potent than maleate; e.g., maleate is 14 times more active than the product with glutathione. These results are puzzling and seem to argue against an SH group reaction,
at least a depletion of the low molecular weight thiols of the cells. Possibly maleate is antimitotic only after reaction with cellular thiols, or is split off from the thiols within the cells and then reacts with some component involved in mitosis, since these tests were run over a period of 24 hr. Further argument against the SH reaction theory is the fact that chloromaleate and chloromaleimide react readily with SH groups but are not antimitotic (Friedmann et al., 1952 a). One might explain this by assuming that the high reactivity of these compounds (chloromaleimide reacts completely with glutathione within 1 min) brings about their immediate inactivation in the cells, the addition products with thiols here being mitotically inactive, as are some of the addition products with the 1,4-naphthoquinones, but one would like some experimental evidence for this. Furthermore, chlorofumarate does not react with thiols and yet is moderately antimitotic.

These potent antimitotic effects on tissue culture cells are not seen with microorganisms. Thus the growth of the fungi Botrytis allii, Fusarium graminearum, and Penicillium digitatum is not inhibited by maleate at concentrations of 3.4–8.6 mM (McGowan et al., 1948), and the minimal bacteriostatic concentrations for Aerobacter aerogenes and Pseudomonas fluorescens are 6.9 mM and 2.5 mM respectively (Cooper and Goddard, 1957). The growth of Staphylococcus aureus and E. coli is not inhibited by 0.84 mM maleate, but maleimide inhibits 50% at 0.011–0.014 mM, indicating that failure of maleate to penetrate into the bacteria might be responsible for its lack of activity (Marrian et al., 1953). The growth of yeast is also very little affected by maleate up to 1 mM (Kiga et al., 1955). The only detailed study of the effects of maleate on bacterial growth is that of Nicolle and Joyeux (1950, 1951). The concentration-inhibition curve for E. coli grown on glucose medium is shown in Fig. 2-3, and it is seen that depression of growth becomes marked only above 30 mM. Maleate is more inhibitory when fumarate is the sole carbon source. One must conclude that bacterial and fungal proliferation does not depend on the same factors as mitosis in fibroblasts.

The investigation of the effects of maleate on plant growth is complicated by the ability of many plant tissues to utilize maleate in their metabolism (see page 312), and stimulation of growth has often been observed. Maleate produces a small but definite stimulation in the bean test for wound hormones (English et al., 1939), increases oat coleoptile extension at 1–5 mM (Albaum and Eichel, 1943), and augments the growth of bean and tomato plants following dipping in 15 mM solutions (Greulach, 1953). No effect by maleate has occasionally been reported, as with wheat roots (Lundegårdh, 1944) and soybean seedlings (Parups et al., 1962) (slight depression noted at 10–50 mM in the latter case). Certainly plant growth does not seem to be sensitive to maleate, and this cannot be entirely explained by failure to penetrate.
The growth of rats is not markedly affected by maleate. Dye et al. (1944) injected doses increasing from 0.5 to 2 mg into young rats for 4 weeks and found the body weight to be about 13% less than in the controls, while Fitzhugh and Nelson (1947) observed growth inhibition in rats over a year when maleate was incorporated at 1–1.5% in the diet. Nothing has been done on the growth of specific proliferating tissues. The marked antimitotic effect in cell cultures brings up the question whether maleate is tumoristatic.

Boyland (1940) found that the administration of 15 mg/day of maleic acid to mice bearing grafted sarcomata and spontaneous carcinomata led to a slight inhibition of tumor growth, the effects being much less than with malonate. Brunschwig et al. (1946) reported very similar results in rats bearing Walker carcino-sarcomata, 300 mg/kg/day for 15 days producing 44% inhibition of tumor growth. There is thus little evidence that maleate is an effective agent in suppressing tumor growth.

MALEAMATE, MALEIMIDE, AND MALEURATE

If the mechanism by which maleate produces some of its metabolic and mitotic effects is through reaction with SH groups, one might expect that chemical changes in the carboxylate groups would not abolish the activity, as it does in the case of malonate which interacts by means of its carboxy-
late groups, and would possibly facilitate penetration into cells. Maleic diamide inhibits soybean seedling growth, minimal effects being observed around 0.1 mM, whereas maleamate (maleic monoamide) is only weakly inhibitory; both, however, are more effective than maleate (Parups et al., 1962). It is not known if these substances react readily with SH groups, but it is likely since maleimide reacts very rapidly with thiols (Friedmann et al., 1949). Maleimide is, however, only a moderately effective antimitotic agent when examined in fibroblast cultures, inhibiting around 22% at 0.005–0.009 mM and producing some delay in metaphase. N-Ethylmaleimide is much more active (see page 364). The impression is that these substances may act differently than maleate, since the phase distribution is not altered in the same way and the cytological changes are not identical.

The antimitotic activity of maleate, maleimide, N-ethylmaleimide, and maleic hydrazide prompted Okada and Roberts (1958) to study maleurate (N-carbamoylmaleamate or monomaleylurea), which combines some of the structural features of the maleate group and glutamine, a substance of interest in tumor metabolism. Onion root tips exposed to 5.7 mM maleurate for 48 hr become flaccid and do not resume growth when placed in fresh water. There is a marked metaphase inhibition (see accompanying tabulation), which is most severe at 10 hr when 61% of the mitotic cells are in metaphase. No abnormal mitoses were observed and it was concluded that the effects do not resemble those of any of the known antimitotic agents. The life-span of mice following inoculation with Ehrlich ascites carcinoma cells is 10–12 days; if the mice are injected with 100–300 mg/kg maleurate, the life-span is not altered at the lower doses and is shortened somewhat at the higher, suggesting insufficient specificity against the tumor cells. However, shortly after injection of maleurate there is blebbing, metaphase clumping, chromosomal stickiness, and numerous micronuclei, cleavage being temporarily blocked but increasing later.

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Maleurate</th>
</tr>
</thead>
<tbody>
<tr>
<td>% in prophase</td>
<td>39</td>
<td>37</td>
</tr>
<tr>
<td>% in metaphase</td>
<td>20</td>
<td>54</td>
</tr>
<tr>
<td>% in anataphase</td>
<td>41</td>
<td>9</td>
</tr>
<tr>
<td>% of cells in mitosis</td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>
Chromatography of tumor cells and ascites fluid showed no free glutamine or changes in amino acid pattern. The LD$_{50}$ of maleurate given intraperitoneally to mice is 775 mg/kg, and 150 mg/kg/day is tolerated for 8 days without evidence of toxicity (Siskin et al., 1959). It was found that a dose of 250 mg/kg almost completely inhibits the incorporation of thymidine-$H^3$ into ascites cells. There is thus interference with DNA formation and a delay in the entrance of interphase cells into mitosis. Various esters of maleurate are more effective than maleurate, perhaps because of better penetration, but the esters affect the formation of the cleavage spindles whereas maleurate does not, so that the mechanisms of action may not be exactly the same (Okada and Roberts, 1963). It was possible to administer doses, which produce abnormalities in the tumor cells without being generally toxic or affecting survival. Maleurate and its derivatives exert very interesting effects on cleavage and it is likely that further investigation will provide some insight into mitotic processes.

**MALEIC HYDRAZIDE**

Maleic hydrazide was introduced in 1949 by the U. S. Rubber Company as a new type of plant growth regulator and herbicide, following the initial report by Schoene and Hoffmann (1949) that this substance produces a unique depression of growth, the inhibition being marked but temporary and often unaccompanied by damage to the plants. If tomato plants are sprayed with 0.2% maleic hydrazide, growth stops for 2 months but the plants remain normal during this time, except for some chlorosis of the lower leaves. There is no terminal growth but the leaves grow to their normal size. Maleic hydrazide has come to be an important commercial herbicide, and much work has been done to elucidate the mechanism by which it so selectively suppresses growth of plants. We shall not discuss the effects on plants in detail; a complete review of this aspect of the subject has been provided by Crafts (1961 a). The treatment here will be confined to those properties possibly relating maleic hydrazide to maleate and reaction with SH groups, and to what little is known of the effects on plant metabolism.

**Structure, Properties, and Reactions**

Maleic hydrazide is commonly written as structure (I), but there is evidence that structures (II) and (IV) may contribute significantly. (1) Maleic hydrazide is a fairly strong acid ($pK_a = 5.65$), which would not be expected from structure (I); however, the resonance form (IV) of the tautomeric structure (II) would account for the lability of a proton (Feuer and Rubinstein, 1958). (2) The ultraviolet spectrum of maleic hydrazide is similar to that of 6-hydroxy-2-methyl-3(2H)-pyridazinone, in-
Maleic hydrazide is soluble in water to the extent of 0.2% (about 18 mM), but as the diethanolamine salt, in which form it is usually applied commercially, it is soluble to at least 2%.

The rapid reaction of maleimide with thiols might lead one to expect maleic hydrazide also to be reactive, but apparently the aromatization of the ring markedly reduces the reactivity. When 5 mM maleic hydrazide is incubated with 0.5 mM glutathione for 5 hr at room temperature, there is no detectable disappearance of SH groups (Leopold and Price, 1957). This is true in the entire pH range 4-7. Furthermore, incubation of maleic hydrazide with thiolacetate, cysteine, or glutathione at 37° and pH 7 for 4 days does not lead to significant loss of SH groups (Weller et al., 1957). No alteration of the SH groups in radish leaf homogenates by maleic hydrazide was observed. Glutathione does not protect phosphorylase against inhibition by maleic hydrazide, indicating that no reaction with glutathione occurs (Hughes and Spragg, 1958). Tests with bromine and permanganate showed the presence of an olefinic bond in maleic hydrazide, and indicated that the C==C bond retains a certain high reactivity characteristic of maleate derivatives, but that this reactivity is not sufficient to allow addition of the SH group, at least in low molecular weight thiols. However, Hughes and Spragg (1958) found that around 60% of the SH groups of β-amylase disappeared after incubation with 4 mM maleic hydrazide, so that possibly certain SH groups on proteins may react more readily than the simple thiols. The statement by Hughes and Spragg, “A reaction could take place between two SH groups and one molecule of maleic hydrazide in such a manner that two protein chains become linked irreversibly” to explain certain mitotic effects, is open to question.

General Effects on Plant Structure and Growth

Maleic hydrazide is able to inhibit the germination of seeds, suppress the growth of roots and terminal shoots, and retard bud and floral develop-
ment. It is somewhat selective in that grasses are generally more susceptible than broad-leaved plants, and young plants are more susceptible than older plants. A few examples will give an idea of the types and potency of the effects. *Vicia faba* roots treated with 0.5 mM maleic hydrazide show no mitoses for 2 days (Darlington and McLeish, 1951), the growth of bean and tomato seedlings is inhibited 85–90% by 15 mM maleic hydrazide used as a dip (Greulach, 1953), the growth of crown gall on carrot roots is suppressed for several days after exposure to 2.67 mM maleic hydrazide (Waggoner and Dimond, 1953), the germination of lettuce seeds is reduced over 90% by 15 mM maleic hydrazide (Haber and White, 1960), the growth of young corn plants is completely stopped by spraying with 8.9 mM maleic hydrazide (Tatum and Curme, 1951), and the growth of moss sporogonia is reduced 50% by 4.4 mM maleic hydrazide (Bopp, 1954).

It has been observed several times that the leaves of treated plants become a darker green or in some cases develop an anthocyanin pigmentation (Currier and Crafts, 1950; Tatum and Curme, 1951; Crafts, 1961 a). Many morphological changes have been reported, including abnormally shaped leaves, disruption of phloem, stomatal degeneration, chloroplast disintegration and reduction, and general stunting. In some instances it appears that mitosis is blocked but cell enlargement is not affected, occasionally resulting in abnormally large cells. One of the best reviews of such changes is by Gifford (1956) who describes the effects of maleic hydrazide on barley seedlings in detail. Three days after spraying there is inhibition of mitoses in the terminal tips, and nuclear aberrations appear in the cells of the protoderm and ground meristem; there is precocious growth of the axillary buds brought about by cell enlargement; by 7 days the cells in the shoot axis have thickened walls, and there is necrosis in the mesophyll parenchyma; after 2 weeks the plants are stunted, and the leaves are thicker greener, and Brittler; finally there is necrosis in the pith and extensive obliteration of the phloem. He compared some of these effects to plant diseases produced by viruses.

Crafts (1961 a) has postulated that perhaps one of the principal effects of maleic hydrazide is a disorganization of the vascular system of the plants, this eventually leading to the necrotic changes seen in the phloem. In this connection, it may be noted that maleic hydrazide alters the transport of Ca++ applied to foliage in apricot trees (Kessler et al., 1961), and it is likely that the uptake of essential ions by the roots is also inhibited. Undoubtedly the primary effect is on mitotic processes in the growing regions of the plant and perhaps on the formation, transport, or utilization of auxins.

**Absorption, Distribution, and Metabolism of Maleic Hydrazide**

Maleic hydrazide enters young growing roots readily and, after foliar application, penetrates into leaves through aqueous pathways; it is not
strongly retained by the tissues and is translocated relatively well, moving freely across mesophyll and along phloem. Indeed, since it is transferred from phloem to xylem so easily, possibly it circulates in plants. Crafts (1961 b) thinks that maleic hydrazide may move along the cell walls of meristematic tissue, as do some dyes, and presents radioautographic evidence for this. If maleic hydrazide is sprayed onto Bermuda grass shoots at 45 mM in solutions of different pH, the maximal growth inhibition occurs at pH 7, relatively little effect being seen above pH 9 or below pH 5, which is difficult to explain satisfactorily on the basis of the ionization of maleic hydrazide. Although the bulk of the absorbed maleic hydrazide may be free to move, the bound fraction is quite stable. Callaghan and Grun (1961) exposed root tips to 1 mM maleic hydrazide-C\textsuperscript{14} for 1 hr and found the initial fixation (first 24 hr) to be mainly nucleolar, but that gradually a shift to the nuclei occurred, so that at 3 weeks the nuclei were well labeled, and Baker (1961) observed that tobacco seedlings treated with maleic hydrazide-C\textsuperscript{14} retain radioactivity in the meristematic regions, the inhibitor being very tightly bound to proteins. Although certain bacteria can utilize maleic hydrazide, its metabolism in plants has not been studied. Parups et al. (1962) examined the activities of the postulated metabolic products of maleic hydrazide on bud formation — maleic diamide, fumaric diamide, maleamate, and maleate — and found that none is nearly as inhibitory as the parent compound.

**Inhibition of Enzymes and Metabolism**

Relatively little work on the effects of maleic hydrazide on enzymes has been done and many important SH enzymes have not been examined, as may be seen in the summary presented in Table 2-7. Furthermore, the results on certain important enzymes, such as succinate oxidase and indoleacetae oxidase, are inconsistent. The inhibition of phosphorylase is not reversed by glutathione but dialysis seems to restore the reacted SH groups in \(\beta\)-amylase (Hughes and Spragg, 1958). Reactivation of enzymes by thiols would not be expected, unless the maleic hydrazide readily dissociates from the enzyme. Nothing is known about the equilibria of the addition reactions of thiols to maleic derivatives. Baker (1961) found that a diaphorase and a nitrate reductase are fairly well inhibited by maleic hydrazide — in fact, to about the same extent as the respiration of tobacco tissues — and postulated that maleic hydrazide might interfere in electron transport systems involving vitamin K.

The pattern of maleic hydrazide action is in some ways similar to that of X-radiation, and since X-radiation causes destruction of auxin, Leopold and Klein (1951) considered the possibility that maleic hydrazide acts as an antiauxin. They showed in the slit pea test that maleic hydrazide inhibits at low auxin concentrations but not when the auxin concentration
### Table 2-7

**Effects of Maleic Hydrazide on Enzymes**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Maleic hydrazide (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldolase</td>
<td>Peas</td>
<td>5</td>
<td>0</td>
<td>Hughes and Spragg (1958)</td>
</tr>
<tr>
<td>$\beta$-Amylase</td>
<td>Barley</td>
<td>4</td>
<td>47</td>
<td>Hughes and Spragg (1958)</td>
</tr>
<tr>
<td></td>
<td>pH 5</td>
<td>4</td>
<td>39</td>
<td></td>
</tr>
<tr>
<td>Glucose-6-P dehydrogenase</td>
<td>Yeast</td>
<td>15</td>
<td>Stim 3</td>
<td>Baker (1961)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Hexokinase</td>
<td>Wheat germ</td>
<td>5</td>
<td>0</td>
<td>Saltman (1953)</td>
</tr>
<tr>
<td>Indoleacetate oxidase</td>
<td><em>Polyporus versicolor</em></td>
<td>1.8</td>
<td>0</td>
<td>Tonhazy and Pelezar (1954)</td>
</tr>
<tr>
<td></td>
<td>Germinating peas</td>
<td>1.5</td>
<td>Stim 90</td>
<td>Andreae and Andreae (1953)</td>
</tr>
<tr>
<td></td>
<td><em>Lens culinaris</em> roots</td>
<td>10</td>
<td>Stim 105</td>
<td>Pilet (1961)</td>
</tr>
<tr>
<td></td>
<td>(young cells)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wheat leaves</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>In dark</td>
<td>0.03</td>
<td>47-67</td>
<td>Waygood and Maelachlan</td>
</tr>
<tr>
<td></td>
<td>In light</td>
<td>0.03</td>
<td>6</td>
<td>(1961)</td>
</tr>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>Snap bean leaves</td>
<td>15</td>
<td>Stim 6</td>
<td>Baker (1961)</td>
</tr>
<tr>
<td>Enzyme/Reaction</td>
<td>Source</td>
<td>Enzyme Activity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malate dehydrogenase (decarboxylating)</td>
<td>Soybean roots</td>
<td>15</td>
<td>Stim 18</td>
<td>Baker (1961)</td>
</tr>
<tr>
<td>NADH:cytochrome e oxidoreductase</td>
<td>Tobacco roots</td>
<td>3</td>
<td>11</td>
<td>Baker (1961)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>15</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>83</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>41</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Phosphorylase</td>
<td>Potato</td>
<td>pH 5.8</td>
<td>5</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH 7.4</td>
<td>5</td>
<td>46</td>
</tr>
<tr>
<td>Succinate oxidase</td>
<td>Onions</td>
<td>4.4</td>
<td>Stim</td>
<td>Isenberg <em>et al.</em> (1954)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Soybean mitochondria</td>
<td>10</td>
<td>9</td>
<td>Switzer (1957)</td>
</tr>
<tr>
<td></td>
<td>Pea seedlings</td>
<td>pH 5</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>pH 7.2</td>
<td>17</td>
<td>Stim 1</td>
</tr>
<tr>
<td></td>
<td>Cucumber hypocotyls</td>
<td>0.3</td>
<td>9</td>
<td>Stemmlid and Saddik (1962)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Trypsin</td>
<td>Pancreas</td>
<td>5</td>
<td>0</td>
<td>Hughes and Spragg (1958)</td>
</tr>
</tbody>
</table>
is increased. The antagonism of maleic hydrazide by auxin was also demonstrated in the *Avena* coleoptile test. Waggoner and Dimond (1953) assumed that a lowering of the auxin level by maleic hydrazide should lead to a suppression of the growth of crown gall due to *Agrobacterium tumefaciens*, and found this to occur without direct effect on the pathogen. Andreae and Andreae (1953) examined the oxidation of indoleacetate in a complex system from germinating peas and observed that 1.5 mM maleic hydrazide markedly stimulates this oxidation. It was postulated that maleic hydrazide might produce some of its growth inhibition by facilitating the destruction of auxin in the tissues. However, subsequent study of the effects of maleic hydrazide on indoleacetate oxidation has not uniformly supported this theory, and in some cases indoleacetate oxidase is inhibited (Table 2-7). These recent results along with actions to be discussed presently make it unlikely that this is an important mechanism.

**Effects on Plant Metabolism**

The possibility that the growth inhibition exerted by maleic hydrazide is related to an inhibition of respiration was examined by Naylor and Davis (1951) in a variety of roots. The mean results from the seven species are shown in the accompanying tabulation. That the inhibition is greater at

<table>
<thead>
<tr>
<th>Maleic hydrazide (mM)</th>
<th>% Inhibition of respiration at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 4</td>
</tr>
<tr>
<td>4.4</td>
<td>24</td>
</tr>
<tr>
<td>8.9</td>
<td>36</td>
</tr>
<tr>
<td>17.8</td>
<td>45</td>
</tr>
</tbody>
</table>

the lower pH is reasonable, since at pH 4 maleic hydrazide is mainly unionized, but the reason for the stimulation usually seen at pH 6 is difficult to explain. Naylor and Davis felt that the respiratory depression might well have something to do with the growth inhibition. The respiration of onion bulbs obtained from plants sprayed with maleic hydrazide is stimulated by low concentrations (around 4.4 mM) and increasingly inhibited up to 35 mM (Isenberg et al., 1954), and this was also taken as evidence that respiratory inhibition is of some significance. The respiration of tobacco leaves is inhibited 10% by 3.3 mM and 44% by 27 mM maleic hydrazide (Callaghan and Van Norman, 1956), and the respiration of tobacco seedlings is similarly reduced, the inhibition progressively increasing with infiltration time up to 4 hr (Baker, 1961). On the other hand, the respiration of wheat seedlings is increased 20-50% by application of maleic
MALEIC HYDRAZIDE

Maleic hydrazide at 10 mg/crock/day (Shaw et al., 1958). It may be mentioned that maleic hydrazide does not inhibit the respiration of mammalian skin at concentrations up to 10 mM (Barnes et al., 1957), which is in conformity with the general lack of toxicity to animals.

Maleic hydrazide retards development of the floral buds of magnolia and the degree of effect is correlated with the quantity of starch in the perianth segments at the time of application (Griesel, 1954). Furthermore, in treated flowers the starch disappears less rapidly than normally, and it was thought that this suppression of starch utilization might explain the respiratory depression observed in other plants. The C-1/C-6 ratio in wheat leaf discs is not altered by maleic hydrazide, although the respiration is somewhat stimulated (Shaw et al., 1958), so we have little evidence for an effect on the pathways of carbohydrate breakdown. The protein content of potatoes obtained from sprayed plants is some 20% greater than in the controls, but during 60-day storage the protein content falls much faster than in the controls (Yasuda et al., 1955). The inhibition of sprouting by maleic hydrazide was thought to be due to this disturbance of protein metabolism. No inhibition of the incorporation of amino acids into protein in pea root mitochondria by maleic hydrazide was observed by Baker (1961). At low concentrations (0.3–1 mM) maleic hydrazide has little effect on either O2 uptake or phosphorylation in cucumber hypocotyl mitochondria, and the P:O ratio is actually elevated slightly (Stemlidl and Saddik, 1962).

The effects of maleic hydrazide on photosynthetic processes are particularly interesting. It has been mentioned that the leaves of plants treated with maleic hydrazide usually become greener, but that there is some reduction of chloroplast number. In Swiss chard sprayed with maleic hydrazide, the number of chloroplasts is decreased by 35–40% while the chloroplast diameter is increased 23% (Callaghan and Van Norman, 1956). Similar changes occur in tobacco plants and here the changes in chlorophyll (chl) content and photosynthesis were investigated (see accompanying tabulation). The photosynthetic mechanism must be affected di-

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Maleic hydrazide at:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3.3 mM</td>
<td>27 mM</td>
<td></td>
</tr>
<tr>
<td>Chlorophyll (mg/cm²)</td>
<td>0.00813</td>
<td>0.00636</td>
<td>0.00933</td>
<td></td>
</tr>
<tr>
<td>Photosynthesis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μliter O₂/cm²/hr)</td>
<td>9.17</td>
<td>12.08</td>
<td>14.42</td>
<td></td>
</tr>
<tr>
<td>(μliters O₂/mg chl/hr)</td>
<td>1128</td>
<td>1928</td>
<td>1545</td>
<td></td>
</tr>
<tr>
<td>Respiration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(μliter O₂/cm²/hr)</td>
<td>1.93</td>
<td>1.74</td>
<td>1.04</td>
<td></td>
</tr>
</tbody>
</table>
rectly since the rate in terms of chlorophyll content is changed. The augmented photosynthesis was considered to be important in the action of maleic hydrazide, but how this could contribute to an inhibition of growth is not clear.

Chromosomal Effects

The similarities between the actions of maleic hydrazide and X-radiation led Darlington and McLeish (1951) to study possible changes in the mitotic processes in *Vicia faba* roots. Low concentrations of maleic hydrazide (0.1 mM) only partially block mitosis but cause chromosomal breakage; at 24 hr there is around 0.47 break per cell. Breakage is most frequent in certain chromosomes and regions, and is confined to the heterochromatin. The chromosomes, however, do not become sticky, as with many other antimitotic agents, and the effects are quite different from those produced by X-radiation. It was suggested that maleic hydrazide might be useful for defining specific genes in the heterochromatic regions. A concentration of 0.2 mM gives a high breakage frequency but allows the subsequent resumption of mitosis and growth (McLeish, 1954). Various factors affecting the chromosomal responses to maleic hydrazide were studied by Kihlman (1956). The number of isolocus breaks in *Vicia* roots increases with the temperature, as does the mitotic inhibition, and decreases with increase in the pH from 4.3 to 7.3, indicating that penetration is a factor here. Removal of O₂ reduces the abnormal metaphase count from 66% to 20% at 24 hr, and both azide and 2,4-dinitrophenol markedly reduce the effects of maleic hydrazide. The absorption of anions by roots depends on oxidative metabolism, and Kihlman felt that the anaerobiosis, azide, and 2,4-dinitrophenol might inhibit the uptake of maleic hydrazide, but also considered the possibility that ATP is involved in chromosomal breakage.

Graf (1957) confirmed the heterochromatic site for the action of maleic hydrazide in corn root cells by showing that the number of anaphase bridges induced is correlated with the number of heterochromatic knobs in the strain of corn used. He also showed that certain derivatives, particularly N-benzoyl maleic hydrazide, are more potent than maleic hydrazide, due possibly to their greater lipid solubility. On the other hand, Dodge (1964) found no chromosomal aberrations or anaphase bridges in the dinoflagellate *Prorocentrum micans* treated with 0.1 mM to 1 mM maleic hydrazide, although nuclear division is strongly inhibited. There is no heterochromatin in the nucleus, but this is not sufficient reason for the lack of effects on the chromosomes.

The superficial structural similarity between maleic hydrazide and pyrimidines such as uracil was noted by Deysson and Deysson (1953), but they could demonstrate no antagonism of maleic hydrazide by uracil in the inhibition of growth and mitosis in onion roots. Biesele (1958) remarked on the structural resemblance of maleic hydrazide to the purines and the
actions of adenine on root tip cells, and concluded that maleic hydrazide might be a pyrimidine or purine antagonist. Callaghan and Grun (1961) noted that maleic hydrazide-\(^{14}\)C becomes fixed in the nuclei of root tip cells and remains there for long periods, and postulated that the incorporation may follow the pathway of RNA synthesis, due to the structural similarity to uracil, the end product being a modified RNA. It would be quite easy to determine if this is the case, but as far as I know it has not been done.

Effects on Animals

It is noteworthy, as Crafts (1961a) has pointed out, that maleic hydrazide is selectively toxic to plants and does not inhibit growth in bacteria, fungi, algae, or animals. The conclusion is that maleic hydrazide affects some process unique to plants. It was also a point of some concern during the early use of maleic hydrazide as a herbicide that it might be carcinogenic, since Darlington and McLeish (1951) remarked that the chromosomal effects observed in plants could, if they occurred in animal cells, lead to tumors. A thorough investigation was made by Barnes et al. (1957), who found that remarkably high doses are tolerated by mammals. The LD\(_{50}\) for rats is 4 g/kg and they are unaffected by a diet containing 1% maleic hydrazide throughout their lifetime. Rats were injected subcutaneously with 500 mg/kg/week for 14 months; of the 52 animals treated, 3 developed sarcomata, and there was none in the controls. Further work was done and both rats and mice were fed a diet 1% in maleic hydrazide for 100 weeks; there was no effect on the growth or health of the animals, and the incidence of tumors was the same in the treated and control groups. Maleic hydrazide applied to mouse skin showed no carcinogenic activity, nor does 1 mM maleic hydrazide affect mitoses in rat ear epidermis, and even 10 mM does not depress the growth of explants from guinea pig ears. No carcinostatic effect in rats bearing Walker tumors was observed. The lack of action in animals cannot be ascribed to a rapid metabolism of maleic hydrazide, since it was shown that 43–62% of the substance administered to rabbits is excreted unchanged within 48 hr. The fate of the remainder is unknown. Mannel and Grice (1957a, b) fed rats a diet containing 2% maleic hydrazide for 6 months and found no effect on the liver with respect to weight, size, number of cells, histology, RNA or DNA content, or phospholipid content, and concluded that maleic hydrazide is not carcinogenic for the liver. The relative lack of effect on organisms other than plants thus seems to be well established, but at present the reason for this selectivity is not understood.
CHAPTER 3

$N$-ETHYLMALEIMIDE

The early work on maleate as an SH reagent by Morgan and Friedmann in Hopkins' laboratory in Cambridge around 1938 was continued by Friedmann and his associates, using the imides of various unsaturated dicarboxylic acids. They established that these imides react much more rapidly than maleate with thiols and are potent antimitotic agents (Friedmann et al., 1949; Marrian, 1949). During the past few years one of the substituted imides, namely $N$-ethylmaleimide, has been used as a selective SH reagent in various fields of research, and it appears that this substance will retain for some time its important place in enzymic and metabolic studies. It is a relatively recent inhibitor and 90% of the reports on its actions have appeared since 1957, 60% in the 4 years between 1960 and 1963. Since 1960 there have been about 30 publications each year on $N$-ethylmaleimide, and this number is at present steadily increasing.

CHEMICAL PROPERTIES

$N$-Ethylmaleimide is one of a number of $N$-substituted maleimides which react readily with thiols. Others members of this group have not been thoroughly investigated or compared with $N$-ethylmaleimide, and quite possibly more useful SH reagents of this type are yet to be found. Since

\[
\text{Maleimide} \quad \text{$N$-Ethylmaleimide} \quad \text{Succinimide}
\]

$N$-ethylmaleimide reacts with SH groups because of its C=C bond, it would be valuable occasionally to compare its effects with those of succinimide or $N$-ethylsuccinimide, these possessing the general structural features of maleimide and $N$-ethylmaleimide without having the ability to react
with thiols. Possibly some of the effects of \(N\)-ethylmaleimide on enzymes and cells are unrelated to reaction with SH groups, and this comparison would be the most direct way of ascertaining the situation. One must remember that \(N\)-ethylmaleimide is a pyrrole derivative (\(N\)-ethyl-2,5-pyrroledione) and consequently could interfere with certain enzymes or in metabolic pathways for this reason.

Commercial \(N\)-ethylmaleimide is apparently pure enough for most purposes and what contaminants occur would not react with SH groups. It may be recrystallized from ethanol. The compound is quite stable in the solid state but in aqueous solution a hydrolytic ring opening leads to

\[
\begin{align*}
\text{HC} & \quad \text{COO}^- \\
\text{NH} \quad \text{CH}_2\text{CH}_3
\end{align*}
\]

\(N\)-Ethylmaleamate

the formation of \(N\)-ethylmaleamate. This reaction occurs to a negligible extent in the pH range below 7 but in alkaline solutions the first order hydrolysis rate increases linearly with the pH (Gregory, 1955). There is a broad absorption band with a maximum near 300 m\(\mu\) for \(N\)-ethylmaleimide and the hydrolysis can be followed by a decrease in absorption at this wavelength. The percentage of hydrolysis in 6 hr is roughly as follows: pH 7—10\%, pH 8—60\%, and pH 9—90\%. Alkaline solutions of \(N\)-ethylmaleimide may be sufficiently stable for short experiments but fresh solutions should be made for each experiment. Reduction of the C=\(\text{C}\) bond to form the succinimide does not occur readily — the polarographic half-wave potentials for several \(N\)-substituted maleimides lie near — 0.8 v (Muir, 1959) — but in biological systems it should not be neglected, although nothing is known of enzyme systems catalyzing this reaction. I have not been able to find the solubility of \(N\)-ethylmaleimide, but solutions up to 200 mM can be prepared.

**Reaction with Thiols**

The unsaturated imides react rapidly with thiols under physiological conditions, yielding products of the following type:

\[
\begin{align*}
\text{R'}-\text{S} & \quad \text{O} \\
\text{HC} & \quad \text{N}\quad \text{R} \\
\text{HC} & \quad \text{O} \\
\text{H}_2\text{C} & \quad \text{CH}_3
\end{align*}
\]

as described by Marrian (1949) for the reactions of \(N\)-ethyl- and \(N\)-phenyl-
maleimides with thiolacetate and o-mercaptobenzoate. The addition product of N-ethylmaleimide and cysteine was first isolated by Smyth et al. (1960) as the water-soluble \( S-(N\text{-ethylsuccinimido})\text{-l-cysteine}. \) However, Friedmann (1952) pointed out that N-ethylmaleimide and glutathione reacting in very low concentration \((0.004 \text{ mM})\) give various products depending on the pH, the buffer, and other factors. The rate of reaction of N-ethylmaleimide with thiols is very fast in most cases, e.g., with cysteine, glutathione, or thiolacetate requiring less than a minute for completion near neutrality (Fig. 3-1), the rate decreasing with decrease of the pH. Similar

![Graph showing reaction rate of N-ethylmaleimide with different pH values.](image)

**Fig. 3-1.** Reaction of N-ethylmaleimide (1 mM) with glutathione (1 mM) at different pH’s. (From Gregory, 1955.)

results have been obtained by Roberts and Rouser (1958) (Fig. 3-2). It may be noted that both the reaction with the SH groups and the spontaneous hydrolysis of N-ethylmaleimide increase with rise in the pH; however, around neutrality the rate of addition of thiols is so much faster than the hydrolysis that the latter becomes relatively insignificant.

N-Ethylmaleimide has been applied to analyses for SH groups. Hanes et al. (1951) and Gutecho and Laufer (1954) showed that the N-ethylmaleimide derivatives of thiols and cysteine peptides can be better separated chromatographically than the parent compounds. Alexander (1958) popularized the spectrophotometric method for thiol assay, using the decrease in the absorption at 300 m\( \mu \) as a measure of SH reaction, and this certainly is the most convenient method, especially for kinetic studies. Concentrations of thiol as low as 0.1 mM can be determined, and the method can to some extent be used in tissue extracts. Benesch et al. (1956) observed that a reddish color appears when the products of the reaction of N-ethyl-
maleimide and homocysteine are made somewhat alkaline, and then found that a number of thiols produce this color, including glutathione and lipoate. This test is rather sensitive, detecting as little as 0.01 mM thiol, and can be used to visualize thiol spots on chromatographic paper, but there is still some doubt as to its complete specificity (see below). Seligman (1954) developed a chromogenic derivative of N-ethylmaleimide for detecting thiols histologically, this being N-(4-hydroxy-1-napthyl)maleimide, which reacts much faster than 2,2'-dihydroxy-6,6'-dinaphthydisulfide (DDD). Tuppy (1959) mentions the yellow N-(4-dimethylamino-3,5-dinitrophenyl)maleimide, which has the advantage that yellow peptide derivatives can be isolated from proteins treated with it. More recently, Clark-Walker and Robinson (1961) have used N-2,4-dinitroanilinomaleimide as a colored reagent and have shown that it can be applied to the labeling of protein SH groups.

The initial addition products of maleimides with thiols sometimes undergo further interesting reactions. The red color observed by Benesch et al. (1956) may be due to polymerization of N-ethylmaleimide in some cases, Smyth et al. (1960) having found a pink crystalline precipitate following the reaction of N-ethylmaleimide and cysteine at pH 7.4, just as in the reaction of N-ethylmaleimide with imidazole. The cysteine which is reacted with the N-ethylmaleimide probably undergoes intramolecular
transamidation to form a thiazane derivative. The product of the reaction of cysteine and the colored N-2,4-dinitroanilinomaleimide undergoes cyclization, involving the amino group of cysteine and resulting in scission of the succinimide ring, the final product being a substituted 1,4-thiazine.

It has been generally supposed that N-ethylmaleimide is specific for SH groups, since no reaction with cystine, oxidized glutathione, and various amino acids had been detected, but Smyth et al. (1960) have shown that under certain conditions (200 mM of reagents and pH 6.4–7.4) imidazole and some amino acids are N-acylated by N-ethylmaleimide. However, in the case of imidazole, there is subsequent hydrolysis to N-ethylmaleamate, which may be further acylated to initiate polymerization. Histidine and some histidine peptides also give this reaction. It is not known if such acylations are of importance in the reactions of N-ethylmaleimide with proteins and enzymes, but it is always possible that certain histidine residues or amino groups on proteins may be in a particularly reactive state. When glycylalanine is incubated with 10 mM N-ethylmaleimide at pH 7.4 and 37° for 2 hr, the amino group adds to the double bond without opening of the imide ring (Smyth et al., 1964). The amino groups of most proteins do not react so readily as shown by comparing the yields of ethylamine (formed from reaction with amino and SH groups) and S-(1,2-dicarboxyethyl)-L-cysteine (formed from reaction with SH groups) from the hydrolysis of proteins previously treated with N-ethylmaleimide. It is pointed out that specificity toward protein SH groups is favored by a pH below neutrality and avoiding excess reagent, but that each protein or enzyme must be treated as a special problem.
3. N-ethylmaleimide

Reaction with Proteins

The SH groups of native proteins are frequently quite unreactive to N-ethylmaleimide and this reagent is not reliable for the titration of total protein SH groups. However, certain accessible SH groups react readily and this provides N-ethylmaleimide with a certain specificity of attack one does not have with the mercurials and many other SH reagents. Indeed, the advantages of N-ethylmaleimide as an SH reagent may be summarized as (1) probable high selectivity for SH groups, (2) reaction with only certain accessible SH groups on enzymes, making possible specific metabolic inhibitions, and (3) reasonably good penetration into cells due to the uncharged nature of the compound. Alexander (1958) did not apply his method for SH group estimation extensively to proteins, but noted that N-ethylmaleimide does not react with all the SH groups and felt that its use would be limited in this field. The reaction with bovine serumalbumin is quite rapid (Fig. 3-2), half-reaction time being around 3 min (Roberts and Rouser, 1958), but essentially no reaction occurs with β-lactoglobulin unless it is denatured with urea or guanidine, in which case reaction is rapid and complete (Habeeb, 1960; Stark et al., 1960). The half-reaction time for denatured β-lactoglobulin is less than 2 min (Leslie et al., 1962 a). The same holds for ovalbumin, the rate being insignificant with the native protein but complete within 5 min if denatured (Leslie et al., 1962 b). Thus proteins, and presumably enzymes, differ markedly in the reactivity of their SH groups.

Human globin reacts with 4.8-5.0 equivalents of N-ethylmaleimide in 10 min, and an additional 0.7-0.8 equivalent is taken up over the next 5 hr, while globin denatured with lauryl sulfate takes up 5.0 equivalents (Cole et al., 1958). Thus globin is a protein which readily reacts with N-ethylmaleimide in both native and denatured states. That the reaction is with SH groups is established by the fact that previous treatment with Hg++ prevents binding of N-ethylmaleimide (Riggs and Wells, 1960). Reaction of hemoglobin with SH reagents alters the oxygen dissociation curves (inhibition of Bohr effect); N-ethylmaleimide reacts much faster with HbO₂ than with Hb, and treatment with N-ethylmaleimide increases the affinity of Hb for oxygen (Riggs, 1961). By using N-ethylmaleimide-C¹⁴ it was possible to show that at least 97% of the radioactivity is associated with the β-chain of hemoglobin and thus the reactive SH groups are in this part of the protein, and consequently it is the β-chain which is involved in the Bohr effect. The effect of N-ethylmaleimide on the oxygenation of hemoglobin may be interpreted in terms of changes in the configurations of the protein chains (R. F. Benesch and R. Benesch, 1962). The initial attachment of N-ethylmaleimide to the reactive hemoglobin SH groups does not alter the Bohr effect or modify the solubility, but a secondary reaction occurs, whereby the bound N-ethylmaleimide interacts with the
groups involved in affecting oxygenation, and as a consequence the succinimide ring is opened to form an \( N \)-ethylsuccinamate derivative (Benesch and Benesch, 1961):

\[
\text{Hb-SH} + \text{HC-\( \equiv \)CO} \rightarrow \text{HC-\( \equiv \)CO} \rightarrow \text{HC-\( \equiv \)CO} \rightarrow \text{HC-CONH-Et}
\]

Since \( N \)-ethylmaleimide is catalytically hydrolyzed in the presence of imidazole in neutral solution, it was postulated that a favorably situated imidazole ring adjacent to the reactive SH group is responsible for the second reaction. Guidotti and Konigsberg (1964) report that \( N \)-ethylmaleimide reacts rather readily with the terminal amino group of the \( \alpha \)-chain but not with the amino group of the \( \beta \)-chain when incubation is carried out at pH 7.15, 25\(^\circ\), and 1.8 mM reagent for 1 hr. Thus certain amino groups can react with \( N \)-ethylmaleimide under conditions often used in enzyme work, and specificity toward SH groups cannot be assumed in all cases.

**INHIBITION OF ENZYMES**

It would be expected on the basis of what is known of the reactions of \( N \)-ethylmaleimide with proteins that the rate of enzyme inhibition would seldom be rapid. The inactivation of xylulose-5-P epimerase proceeds steadily over 40 min and would probably continue further (Tabachnick et al., 1958), and alcohol dehydrogenase is inhibited even more slowly (Witter, 1960). Phosphoglucomutase was found to be inhibited by \( N \)-ethylmaleimide more slowly than by any of the other SH reagents, around 50% inhibition being seen in 25 min and complete inhibition at 2 hr (Mistelstein, 1961). It requires 90 min to achieve full inactivation of penicillinase by \( N \)-ethylmaleimide, but \( p \)-MB takes only 10 min (J. T. Smith, 1963 a). In some instances a certain degree of inhibition is reached rapidly and further inhibition occurs slowly, as with lipase, 21% inhibition being observed after 15 min and only 47% after 18 hr (Ory et al., 1960). The rate of inhibition depends strongly on pH, as expected. Adenylate kinase requires 30 min to be maximally inactivated by 0.2 mM \( N \)-ethylmaleimide at pH 7.5, but at pH 9 a concentration of 0.05 mM gives the same inactivation in 5 min (Gregory, 1955). The situation with penicillinase is confusing, since, J. T. Smith (1963 a) stated that \( N \)-ethylmaleimide is more effective at pH 7.4 than at pH 6, but in another report (J. T. Smith, 1963 b) gave data indicating that it was about 10 times more inhibitory at pH 6 than at pH 7.4. If the inhibition is due to reaction with enzyme SH groups, one would anticipate an increased effectiveness with an increase in the pH.
In any event, enzyme inhibition with N-ethylmaleimide is generally slow and more attention should be paid to the kinetics in studies using this substance to detect SH groups on enzymes, and it is likely that some of the failures to observe inhibition are due to insufficient incubation time. Thus the data given in Table 3-1 must be used with some caution. In most cases the inhibition by N-ethylmaleimide is probably irreversible, although this has not been commonly investigated. The inhibition of leucine aminopeptidase is not reversed by cysteine (Green et al., 1955), and the inhibition of glutathione reductase is not reversed by dialysis (Mapson and Isherwood, 1963).

N-Ethylmaleimide sometimes reacts with SH groups which are at or near the binding sites for coenzymes or other components of the enzyme reaction. Incubation of the L(+)--lactate dehydrogenase of yeast with N-ethylmaleimide leads to some dissociation of flavin from the enzyme (Armstrong et al., 1960), and the binding of NADH to cytochrome b$_5$ aporeductase is almost completely prevented by 0.02 mM N-ethylmaleimide (Strittmatter, 1961 b), although the binding of FMN is not affected. Other evidence comes from protection experiments. NAD and NADH partially protect the K$^+$-activated aldehyde dehydrogenase of yeast against N-ethylmaleimide, while NADP protects the NADP-linked aldehyde dehydrogenase (Stoppani and Milstein, 1957 b). NADH also protects the liver alcohol dehydrogenase quite effectively (Witter, 1960). The fatty acid-activating enzyme is protected partially by ATP (Jencks and Lipmann, 1957), and succinate dehydrogenase is protected to some extent by phosphate (Stoppani and Brignone, 1956). One cannot certainly conclude that the protector is bound to the SH groups which are reacted by N-ethylmaleimide, but it is likely that the SH groups are at least close to the binding sites. The inhibition of glutaminase by N-ethylmaleimide was stated by Sayre and Roberts (1958) to be more nearly competitive with glutamine than noncompetitive, and the $1/v - [1/(S)]$ plot, despite some nonlinearity of the curve for the inhibited enzyme, certainly indicates a mixed inhibition, which is probably the result of the nonequilibrium conditions (i.e., the glutamine probably is exerting a protective action and delaying the development of the inhibition, the reactive SH group being vicinal to the glutamine site).

Glutathione reductase presents an interesting situation since incubation of the enzyme with N-ethylmaleimide in the absence of NADH or NADPH does not result in inhibition, whereas the presence of NADPH allows the N-ethylmaleimide to react with the enzyme (Black and Hudson, 1961). It was suggested that NADPH must expose one or more SH groups when it is bound to the enzyme, and it was demonstrated with N-ethylmaleimide-C$^{14}$ that more of the inhibitor is attached to the enzyme when NADPH is present. The SH groups exposed are not those of the lipoate
residue. When the enzyme and N-ethylmaleimide are incubated alone, no inhibition occurs, but when NADPH is added, inhibition occurs progressively; when N-ethylmaleimide and NADPH are added together, inhibition is rapid and marked (80-90% in 10 min from 0.02 mM) (Mapson and Isherwood, 1963). There is no reaction of N-ethylmaleimide with the NADPH but with an enzyme group evoked by the NADPH.

N-Ethylmaleimide has been used with varying success to titrate enzyme SH groups. The first work of this type was by Krimsky and Racker (1954) in efforts to demonstrate the presence and function of glutathione in glyceraldehyde-3-P dehydrogenase. Tryptic digestion of the enzyme releases glutathione but if the enzyme is pretreated with N-ethylmaleimide the yield of glutathione is very low, indicating that the bound glutathione has reacted with the reagent. Thetin-homocysteine methyltransferase is inhibited progressively by N-ethylmaleimide and simultaneously there is a reduction in the number of free SH groups (Durell and Cantoní, 1959). The activity decreases more rapidly than the free SH groups, however, in contrast to the results with p-MB, and this could be due either to a secondary structural alteration of the enzyme or to the fact that all the SH groups are not involved in the enzyme activity. Microsomal cytochrome c reductase reacts rapidly with N-ethylmaleimide and is completely inactivated when two SH groups have disappeared, a third SH group reacting more slowly (P. Strittmatter, 1959). One of the two reactive SH groups can be protected by NADH. Ficin also reacts completely with N-ethylmaleimide at pH 6.8, one SH group being accessible in the native enzyme and two SH groups in the denatured enzyme (Liener, 1961). Sweet potato β-amylase has four SH groups which react readily with N-ethylmaleimide at pH 8 but do not at pH 4.5 (Speck et al., 1961). Reaction of one SH group leads to 50% inhibition, so that one critical SH group may be involved, but the inhibition never becomes complete even with reaction of all the SH groups. They followed the reaction by disappearance of N-ethylmaleimide and also by determination of S-cysteininosuccinate in the hydrolyzates of the enzyme, and since these data always checked it seems likely that only SH groups are attacked. The some seven SH groups of lactate dehydrogenase, however, do not react at all with N-ethylmaleimide (Pfleiderer et al., 1958). Summarizing the titration work, one must conclude that N-ethylmaleimide has proved itself to be a rather effective agent, especially when used in conjunction with other SH reagents.

**Effects on ATPase and Actomyosin**

The SH groups of actin are 40-50% titrated with N-ethylmaleimide but the reactivity is somewhat less than with myosin (Tsao and Bailey, 1953). If actin is treated with 2.5 mM N-ethylmaleimide for 20 min the ability to undergo the G-actin → F-actin transformation is not impaired, although
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>$N$-Ethylmaleimide (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylenemonomocarboxylate hydrase</td>
<td><em>Pseudomonas</em> sp.</td>
<td>1</td>
<td>0</td>
<td>Yamada and Jakoby (1959)</td>
</tr>
<tr>
<td>Acetylenedicarboxylate decarboxylase</td>
<td><em>Pseudomonas</em> sp.</td>
<td>1</td>
<td>14</td>
<td>Yamada and Jakoby (1958)</td>
</tr>
<tr>
<td>Acyl-CoA carboxylase</td>
<td>Pig heart</td>
<td>2</td>
<td>100</td>
<td>Kupiecki and Coon (1959)</td>
</tr>
<tr>
<td>Adenylosuccinate lyase</td>
<td>Yeast</td>
<td>0.75</td>
<td>80</td>
<td>Cohen and Bridger (1964)</td>
</tr>
<tr>
<td>Aldehyde dehydrogenase</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAD-specific</td>
<td>Liver</td>
<td>0.18</td>
<td>70</td>
<td>Stoppani and Milstein (1957 a)</td>
</tr>
<tr>
<td>NADP-specific</td>
<td>Yeast</td>
<td>0.02</td>
<td>99</td>
<td>Stoppani and Milstein (1957 a)</td>
</tr>
<tr>
<td>K⁺-activated</td>
<td>Yeast</td>
<td>0.024</td>
<td>50</td>
<td>Stoppani and Milstein (1957 a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.048</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Alliin lyase</td>
<td><em>Allium cepa</em></td>
<td>0.1</td>
<td>0</td>
<td>Kupiecki and Virtanen (1960)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Aminoacylase</td>
<td><em>Brassica campestris</em></td>
<td>3</td>
<td>Stim 3</td>
<td>Ozaki and Wetter (1961)</td>
</tr>
<tr>
<td>Aminopeptidase</td>
<td>Beef lens</td>
<td>0.2</td>
<td>50</td>
<td>Wolff and Resnik (1963)</td>
</tr>
<tr>
<td>α-Amylase</td>
<td><em>Bacillus stearothermophilus</em></td>
<td>10</td>
<td>0</td>
<td>Manning and Campbell (1961)</td>
</tr>
<tr>
<td>Apyrase</td>
<td>Potato</td>
<td>5</td>
<td>Stim 6</td>
<td>Bárány and Bárány (1959 b)</td>
</tr>
<tr>
<td>Arginine kinase</td>
<td>Crayfish muscle</td>
<td>0.01</td>
<td>10</td>
<td>Morrison <em>et al.</em> (1957)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>85</td>
<td></td>
</tr>
<tr>
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<td>Ox cornea</td>
<td>0.2</td>
<td>Stim 20</td>
<td>Wortman (1962)</td>
</tr>
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<td>Arylsulfatase $b$</td>
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<td>Wortman (1962)</td>
</tr>
<tr>
<td></td>
<td>Rabbit cornea</td>
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<td>0</td>
<td>Wortman (1962)</td>
</tr>
<tr>
<td>Enzyme</td>
<td>Source</td>
<td>Specific Activity</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>---------------------------------------</td>
<td>-------------------------------</td>
<td>-------------------</td>
<td>--------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Arylsulfatase d</td>
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<td>Wortman (1962)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rabbit cornea</td>
<td>0.2</td>
<td>Wortman (1962)</td>
<td></td>
</tr>
<tr>
<td>d-Asparaginase</td>
<td><em>Brucella abortus</em></td>
<td>1.67</td>
<td>Altenbern and Housewright (1954)</td>
<td></td>
</tr>
<tr>
<td>l-Asparaginase</td>
<td><em>Brucella abortus</em></td>
<td>1.67</td>
<td>Altenbern and Housewright (1954)</td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>Bacillus coagulans</em></td>
<td>0.01</td>
<td>Manning and Campbell (1957)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>O'Brien and Campbell (1957)</td>
</tr>
<tr>
<td></td>
<td>Beef lens</td>
<td>0.13</td>
<td>0</td>
<td>van Heyningen and Waley (1963)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.3</td>
<td>87</td>
<td></td>
</tr>
<tr>
<td>Protein disulfide reductase</td>
<td>Yeast</td>
<td>0.5</td>
<td>42</td>
<td>Asahi <em>et al.</em> (1961)</td>
</tr>
<tr>
<td>l-Pyrroline-5-carboxylate reductase</td>
<td>Beef liver</td>
<td>0.1</td>
<td>27</td>
<td>Adams and Goldstone (1960 b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.25</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Pyruvate carboxylase</td>
<td>Chicken liver</td>
<td>5.4</td>
<td>19</td>
<td>Keech and Utter (1963)</td>
</tr>
<tr>
<td>Pyruvate dehydrogenase</td>
<td><em>Mycobacterium tuberculosis</em></td>
<td>6</td>
<td>80</td>
<td>Goldman (1960)</td>
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<tr>
<td>Ribonuclease</td>
<td>Beef pancreas</td>
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<td>Rabinovitch and Barron (1955)</td>
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<tr>
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<td>Dickman <em>et al.</em> (1956)</td>
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<td>84</td>
<td>Tabachnick <em>et al.</em> (1958)</td>
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<tr>
<td></td>
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<td>87</td>
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<td></td>
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<td>13</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Sedoheptulose-1,7-diphosphatase</td>
<td>Human erythrocytes</td>
<td>5</td>
<td>95</td>
<td>Urivetzky and Tsuboi (1963)</td>
</tr>
<tr>
<td>Serine deaminase</td>
<td>Yeast</td>
<td>1</td>
<td>0</td>
<td>Racker and Schroeder (1958)</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>Rat liver</td>
<td>1</td>
<td>90</td>
<td>Selim and Greenberg (1960)</td>
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<tr>
<td></td>
<td>Pig heart</td>
<td>0.36</td>
<td>100</td>
<td>Stoppani and Brignone (1956)</td>
</tr>
<tr>
<td>Succinate oxidase</td>
<td>Rat liver</td>
<td>5</td>
<td>100</td>
<td>Nachlas <em>et al.</em> (1960)</td>
</tr>
<tr>
<td></td>
<td>Mouse stomach</td>
<td>1</td>
<td>33</td>
<td>Davenport <em>et al.</em> (1956)</td>
</tr>
<tr>
<td>Enzyme Name</td>
<td>Source</td>
<td>Ki (M)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>-------------------</td>
<td>--------</td>
<td>-----------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Succinate semialdehyde dehydrogenase</td>
<td>Monkey brain</td>
<td>0.01</td>
<td>Albers and Koval (1961)</td>
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</tr>
<tr>
<td>Tetrahydrofolate dehydrogenase</td>
<td>Yeast</td>
<td>0.1</td>
<td>Ramaasatri and Blakley (1962)</td>
<td></td>
</tr>
<tr>
<td>Thetin-homocysteine methylferase</td>
<td>Horse liver</td>
<td>40</td>
<td>Durell and Cantoni (1959)</td>
<td></td>
</tr>
<tr>
<td>Thiamine diphosphatase</td>
<td>Rat liver</td>
<td>0.1</td>
<td>Tilander and Kiessling (1961)</td>
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<td>Thiol transacylase</td>
<td>Clostridium kluyveri</td>
<td>0.42</td>
<td>Alberts et al. (1963)</td>
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<td></td>
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<td></td>
<td>4.2</td>
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<td>1-Threonine deaminase</td>
<td>Sheep liver</td>
<td>0.1</td>
<td>Nishimura and Greenberg (1961)</td>
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</tr>
<tr>
<td>Threonine dehydrogenase</td>
<td>Bullfrog liver</td>
<td>0.05</td>
<td>Hartshorne and Greenberg (1964)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transaminases</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>γ-Aminobutyrate:α-ketoglutarate</td>
<td>Rat liver</td>
<td>0.1</td>
<td>Baxter and Roberts (1958)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspartate:α-ketoglutarate</td>
<td>Beef brain</td>
<td>1</td>
<td>Goldstone and Adams (1962)</td>
<td></td>
</tr>
<tr>
<td>Transketolase</td>
<td>Yeast</td>
<td>—</td>
<td>Datta and Racker (1961)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pig liver</td>
<td>0.1</td>
<td>Simpson (1960)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UDPgalactose pyrophosphorylase</td>
<td>Calf liver</td>
<td>0.1</td>
<td>Isselbacher (1958)</td>
<td></td>
</tr>
<tr>
<td>Xylose isomerase</td>
<td>Pseudomonas hydrophila</td>
<td>0.1</td>
<td>Hochster (1955)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>d-Xyulokinase</td>
<td>Aerobacter aerogenes</td>
<td>1</td>
<td>Bhuyan and Simpson (1962)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xylulose-5-P epimerase</td>
<td>Rabbit muscle</td>
<td>11</td>
<td>Tabachnick et al. (1958)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>65</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
half the SH groups are reacted, whereas p-MB effectively inhibits this transformation. Furthermore, N-ethylmaleimide does not significantly alter the viscosity or the Ca\textsuperscript{++}-binding power of actin (Bárány et al., 1962). The lack of effect of N-ethylmaleimide on the polymerization of actin was confirmed by Tonomura and Yoshimura (1962) and Drabikowski and Gergely (1963). There are apparently at least three classes of SH groups in actin; one type is involved in polymerization, one type in ATP binding, and the remainder do not function in either. It seems that only the third type reacts with N-ethylmaleimide. Katz and Mommaerts (1962) found that two of the total of six SH groups of actin react readily with N-ethylmaleimide, the other four reacting only if the protein is denatured.

![Graph](image_url)

Fig. 3.3. Effects of N-ethylmaleimide on myosin ATPase at pH 7.2 and after an exposure of 2 hr. (Modified from Kielley and Bradley, 1956.)

Myosin SH groups react with N-ethylmaleimide at a rate dependent on the pH but only a fraction is accessible (Kielley and Bradley, 1956). Inhibition of the EDTA-activated ATPase occurs readily and completely, at a time when about one fourth the SH groups have reacted, but the Ca\textsuperscript{++}-activated ATPase activity is only augmented by N-ethylmaleimide (Fig. 3-3). This situation is similar to the response to mercurials (Fig. II-7-23). Blum (1962 a, b) found a pattern of activation and inhibition of the ATPase activity of actomyosin, with respect both to time and to concentration
of N-ethylmaleimide; e.g., 5.5 moles of N-ethylmaleimide/10 g actomyosin at pH 7.5 cause activation up to 20 min but this is then followed by depression. It is interesting that ATP protects the ATPase activity against N-ethylmaleimide, whereas ITP increases the loss of ITPase activity, this indicating that ATP and ITP interact differently with myosin. The pyrophosphate-binding site is protected from N-ethylmaleimide by pyrophosphate, ADP, and 2,4-dinitrophenol, but the ATPase site is not protected (Martonosi and Meyer, 1964). Using N-ethylmaleimide-C¹⁴, Sekine et al. (1962) studied the kinetics of the reaction of myosin SH groups.

Native myosin reacts fairly slowly, whereas after denaturation by guanidine the rate is high (Fig. 3-4). The ATPase activity is completely lost when two SH groups per subunit of myosin are reacted. N-Ethylmaleimide is more selective than the mercurials, although it reacts slower, in that with mercurials half titration leads to only 70–75% inhibition. The myosin treated with the labeled N-ethylmaleimide was hydrolyzed and the peptides examined chromatographically, and it was demonstrated that the cystein residues are attacked.

The ATPase activity associated with a fraction from ox brain (supposed to be in the cell membranes and involved with ion transport) is inhibited by N-ethylmaleimide, but the Mg²⁺-activated and Mg²⁺—Na⁺—K⁺-activated components respond somewhat differently (Skou, 1963). The former
is inhibited more rapidly, but eventually the latter is more depressed (see accompanying tabulation). ATP protects against \( N \)-ethylmaleimide when

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Mg(^{++})-activated</th>
<th>Mg(^{++})-Na(^{+})-K(^{+})-activated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>52</td>
<td>28</td>
</tr>
<tr>
<td>10</td>
<td>72</td>
<td>70</td>
</tr>
<tr>
<td>60</td>
<td>82</td>
<td>91</td>
</tr>
</tbody>
</table>

all the ions are present but does not protect the Mg\(^{++}\)-activated ATPase. This preparation also contains NADH:cytochrome c oxidoreductase activity and this inhibited strongly by \( N \)-ethylmaleimide. It was concluded that there are at least three different types of SH group: (1) those required for Na\(^{+}\)-K\(^{+}\) activation, these reacting slowly, (2) those required for oxidoreductase activity, these reacting rapidly, and (3) those required for Mg\(^{++}\) activation, these reacting rapidly with \( N \)-ethylmaleimide and not being protected by ATP or NADH.

**EFFECTS ON METABOLISM**

Only superficial information for the most part is available on the metabolic effects of \( N \)-ethylmaleimide, and in no case has the site of action been localized at a particular enzyme. Furthermore, many of the critical enzymes in the major metabolic pathways have not been studied, as inspection of Table 3-1 makes evident. We find, for example, that respiration of \textit{E. coli} is inhibited 60\% by 1 mM (Bridges, 1961), of sunflower stems around 10\% by 1 mM (Niedergang-Kamien and Leopold, 1957), and of human erythrocytes some 50\% by 1.3 mM \( N \)-ethylmaleimide (Sheets and Hamilton, 1958) but one has no basis for attributing these inhibitions to any enzyme or group of enzymes. At these concentrations certain dehydrogenases, electron transport systems, glycolytic and cycle enzymes are inhibited, and the over-all respiratory inhibition may be related to all these sites; on the other hand, it is possible, in view of our ignorance, that a particular enzyme is selectively affected. \( N \)-Ethylmaleimide has the potentiality for being a selective inhibitor but we do not know if it is. One of the most likely points of attack might be the \( \alpha \)-keto acid oxidases, since the oxidation of \( \alpha \)-ketoglutarate by liver and kidney mitochondria is quite potently inhibited (Table 3-1) (Rogulski et al., 1962). Another likely site is the glycolytic pathway, since \( N \)-ethylmaleimide has been shown to depress glycolysis in stomach (Davenport et al., 1955), yeast (Weitzen and
EFFECTS ON METABOLISM

Buddecke, 1959), and duck erythrocytes (Tosteson and Johnson, 1957). This inhibition is quite potent, anaerobic glycolysis in yeast being inhibited 50% by 0.13 mM N-ethylmaleimide (about twice as potent as iodoacetate). In erythrocytes the uptake of glucose is depressed even more than glycolysis, being essentially stopped at 1 mM. The synthesis of cellulose by \textit{Acetobacter xylinum} is completely blocked by 2 mM-ethylmaleimide, but whether this is an action on glucose uptake or on the polymerization is not known (Schramm et al., 1957).

Lipid biosynthesis seems to be quite sensitive to N-ethylmaleimide. The formation of fatty acids from acetyl-CoA and malonyl-CoA by an extract of pigeon liver is reduced 56% by 0.012 mM and 93% by 0.025 mM (Bressler and Wakil, 1962). It would be worthwhile to know how readily N-ethylmaleimide reacts with coenzyme A. Sterol synthesis can be blocked at two sites at least, the formation of squalene from farnesyl-PP (Goodman and Popják, 1960) and the conversion of squalene to sterols (Goodman, 1961), both pathways being inhibited around 50% by 0.3–1 mM N-ethylmaleimide. Much less is known of the effects of N-ethylmaleimide on protein synthesis, but in rabbit reticulocytes even 1 mM has scarcely any effect on the incorporation of labeled leucine into protein (Borsook et al., 1957).

The uptake of cytidine into HeLa cell RNA is moderately reduced by N-ethylmaleimide at 0.02–0.2 mM (Srinivasan et al., 1964). The exact site of the inhibition is not known but the RNA polymerase was suggested. Another site in nucleoside metabolism was located by Younathan et al. (1964) in the synthesis of UTP from uracil by extracts of rat brain, this being 99% suppressed by 0.1 mM N-ethylmalaimide. They showed that the mechanism does not involve depletion of ATP or complexing with Mg++, and since uridine and UMP accumulate it was assumed that the primary attack is on the ATP:UMP phosphotransferase. This is substantiated by the lack of inhibition on uridine phosphorylase and uridine kinase. The incorporation of $^{59}$Fe++ into ferritin in liver slices is inhibited 90% by 5 mM N-ethylmaleimide (Mazur et al., 1960). Bioluminescence in the sea pansy Renilla reniformis is rather potently depressed by N-ethylmaleimide, 31% inhibition occurring at a concentration of 0.1 mM (Cormier, 1960). Out of such isolated strands no significant pattern can be woven.

Insulin facilitates the uptake of glucose by tissues and it has been postulated that insulin is bound to the cell membranes by a disulfide link. Cadenas et al. (1961) used N-ethylmaleimide to block the SH groups at the cell surface which may be involved in insulin binding. The isolated rat heart takes up glucose at a slow rate and the addition of insulin to the perfusion fluid augments this uptake. Perfusion for 30 sec with 1 mM N-ethylmaleimide does not affect the basal glucose uptake but inhibits the insulin-stimulated uptake by 80%. If the hearts are pretreated with insulin, the inhibition by N-ethylmaleimide is prevented, which may be interpreted
as receptor occupancy by the insulin. Finally, the binding of insulin-I$^{131}$ to hearts is reduced to half by $N$-ethylmaleimide. All of these results could be adequately explained by assuming that $N$-ethylmaleimide reacts with those membrane SH groups involved in insulin binding, although they would not prove that a disulfide bond is formed. The studies of Mirsky and Perisutti (1962) on rat epididymal adipose tissue gave very different results. $N$-Ethylmaleimide at 1 mM inhibits the basal glucose oxidation 70% and the insulin-stimulated oxidation 81%. It may well be that a true differential effect on uptake is obscured by the fact that measurements of the C$^{14}$O$_2$ produced from labeled glucose were made. Furthermore, binding of insulin-I$^{131}$ is not affected by 1 mM $N$-ethylmaleimide in either adipose tissue or diaphragm. Ott and Recant (1963) confirmed the results on adipose tissue; on the other hand, one sees in the data that the effect of insulin is progressively suppressed as the concentration of $N$-ethylmaleimide rises, being a complete inhibition at 1 mM; e.g., normally 500 $\mu$units/ml of insulin stimulate glucose oxidation 790% whereas in the presence of 1 mM $N$-ethylmaleimide there is only a 60% increase. They could also detect no effect of $N$-ethylmaleimide on the binding of insulin. The problem thus rests in this unsatisfactory state and one does not know whether to credit the discrepancies to tissue differences or to variations in the techniques.

**EFFECTS ON TISSUE FUNCTIONS**

The use of $N$-ethylmaleimide to investigate the metabolic basis for tissue function has been very limited, but the results obtained suggest that it deserves more extensive consideration as a possibly selective SH reagent, especially for the study of active transport.

**Gastric Acid Secretion**

$N$-Ethylmaleimide, in common with other SH reagents, effectively inhibits the secretion of acid by the parietal cells (Davenport et al., 1955). Indeed, $N$-ethylmaleimide is about 4 times more potent than iodoacetamide. Stimulation of the secretion is observed at low concentrations, as with iodoacetamide (Fig. 3-5). Both the resting and the carbachol-stimulated secretion are depressed. Hollander (1956) showed that these *in vitro* results can also be obtained *in vivo* by applying 1.5–2 mM solutions of $N$-ethylmaleimide topically to Heidenhain pouches in the dog. The histamine-stimulated acid secretion is blocked for long periods but recovery eventually occurs after 12–24 hr.

Glycolysis in the stomach is inhibited by $N$-ethylmaleimide and it appears superficially that this may be correlated with the secretory suppres-
sion, but if acetoacetate is used as a substrate instead of glucose the inhibition of acid secretion remains (Davenport et al., 1955). When $N$-ethylmaleimide is used in conjunction with 2,4-dinitrophenol in the technique previously described (page 1-504), it was found that the inflection point in the concentration-inhibition curve for the uncoupler is shifted somewhat to the left, i.e., $N$-ethylmaleimide augments the action of 2,4-dinitrophenol (about one third the concentration required for minimal inhibition), indicating the primary effect of $N$-ethylmaleimide to be on some process of ATP formation rather than on ATP utilization. If mouse stomachs are incubated for 10 min with 1 mM $N$-ethylmaleimide, acid secretion is reduced 80%, and simultaneously there is a 33% inhibition of succinate oxidase (Davenport et al., 1956). However, it was felt that this is not an important site of action and is not the cause of the secretory inhibition. Nevertheless, it may well indicate an appreciable inhibition of the operation of the cycle, inasmuch as succinate oxidase may not be as sensitive to the inhibitor as the $\alpha$-keto acid oxidases, and this could contribute to the interference with ATP generation. Davenport believed that the SH groups important for acid secretion are not involved in oxidative systems, but rather are on some specific portion of the secretory system. The evidence for this comes mainly from work on iodoacetamide, but there is some doubt if $N$-ethylmaleimide acts in exactly the same way since the effects on the susceptibility to 2,4-dinitrophenol are quite opposite. An attempt was made to determine if the critical SH groups are those of glutathione, coenzyme A, or lipoate, and it was found that the concentrations of all

![Fig. 3-5. Effect of $N$-ethylmaleimide on acid secretion from mouse gastric mucosa after 10-min exposure. (From Davenport et al., 1955.)](image-url)
three are quite markedly reduced by incubation with \( N \)-ethylmaleimide (see accompanying tabulation). Lipoate is reduced only by concentrations

<table>
<thead>
<tr>
<th>( N )-Ethylmaleimide</th>
<th>( % ) Reduction in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glutathione</td>
</tr>
<tr>
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<td>3</td>
</tr>
<tr>
<td>0.25</td>
<td>30</td>
</tr>
<tr>
<td>0.5</td>
<td>50</td>
</tr>
<tr>
<td>1</td>
<td>69</td>
</tr>
<tr>
<td>2</td>
<td>84</td>
</tr>
</tbody>
</table>

above 1 \( mM \) \( N \)-ethylmaleimide so it can perhaps be eliminated as a factor in the secretory inhibition. Addition of glutathione does not modify the inhibition and it was believed that this is evidence for discounting the importance of glutathione, but possibly the glutathione reacted is tightly bound to some enzyme or transport system and cannot be easily replaced. Slight recovery occurs when coenzyme A is added to inhibited stomachs but only at the lowest \( N \)-ethylmaleimide concentrations, and the results were not considered sufficiently significant to be indicative of the participation of coenzyme A. However, the reduction in coenzyme A and the secretory inhibition proceed in parallel so that at least part of the inhibition may be related to the inactivation of coenzyme A. It may be noted that iodoacetamide does not reduce coenzyme A parallel to secretory inhibition, substantiating a difference in the sites of action of these inhibitors. Probenecid inhibits certain renal transports and has been suggested to react with coenzyme A. It was found that probenecid above 0.1 \( mM \) inhibits acid secretion and that 0.5 \( mM \) \( N \)-ethylmaleimide lowers the probenecid threshold by a factor of 80. These results are consistent with an action of \( N \)-ethylmaleimide on coenzyme A. The problems and limitations inherent in such analyses of inhibitor action are well illustrated by this work and the original papers will repay careful reading by those interested in using inhibitors to determine the mechanisms of cellular function.

**Axonal Conduction and Ganglionic Transmission**

H. M. Smith (1958) has shown that \( N \)-ethylmaleimide at 2 \( mM \) blocks the conduction in frog sciatic nerves in 21 min, and at 1 \( mM \) blocks conduction in the lobster giant axon in 10 min. Conduction blockade occurs long before depolarization of the axons — in the case of the lobster nerve it requires 90 min to depolarize — and this might suggest that \( N \)-ethylmaleimide exerts some action other than depolarization, although the latter
must contribute to some extent. The results were discussed in terms of
the role of SH groups in the axonal membrane structure and the generation
of the action potential.

The injection of 0.5 mg of N-ethylmaleimide into the carotid artery
brings about marked changes in the transmission across the cat cervical
sympathetic ganglion (Komalahiranya and Volle, 1963). The effects are
divided chronologically into 3 phases:

Phase I (0–10 min): The amplitude of the postganglionic action potential
is somewhat increased but postganglionic firing induced by injections of
acetylcholine or K+ is completely blocked. Denervated ganglia treated
with N-ethylmaleimide also lose their response to acetylcholine and K+.

Phase II (10–45 min): The postganglionic response to preganglionic
stimulation is reduced and occasionally blocked irreversibly. At this time
the postganglionic responses to acetylcholine and K+ are markedly en-
hanced. Retrograde firing induced by K+ is also enhanced.

Phase III (45–120 min): Spontaneous asynchronous discharges occur
in both pre- and postganglionic nerves and this is not prevented by the
usual ganglionic blockers.

The initial enhancement of the postganglionic spike they attribute to a
preganglionic site of action, possibly an increase in the release of acetyl-
choline, and the transmission block later to a postganglionic action, which
is nonspecific since it applies to both acetylcholine and K+. The early and
late phases are similar to the effects of high and low Ca++, respectively,
and it is suggested that N-ethylmaleimide may disturb Ca++ balance in
some way, possibly initially releasing Ca++ from extraneural sites and
thereby increasing Ca++ concentration in the ganglion, and eventually
depleting Ca++ from the ganglion itself. They also point out that choline
acetylase is an SH enzyme and might be inhibited by N-ethylmaleimide,
this contributing to the block by reducing the synthesis of acetylcholine.

Erythrocyte Permeability, Active Transport, and Hemolysis

The effects of the mercurials and N-ethylmaleimide on erythrocytes are
quite different. Whereas p-MB readily produces hemolysis in a predictable
manner, N-ethylmaleimide does not; indeed, no hemolysis of human
erthrocytes occurs after 5 hr in 0.5 mM N-ethylmaleimide (Sheets et al.,
1956 b). The respiration of erythrocytes is inhibited 50% by 1.3 mM and
completely by 2.6 mM N-ethylmaleimide, so this inhibitor can affect
metabolism without disrupting the membrane structure, which p-MB
cannot do (Sheets and Hamilton, 1958). Furthermore, N-ethylmaleimide
is much more effective than p-MB in reducing erythrocyte glutathione
(see accompanying tabulation) (Tsen and Collier, 1960) and yet hemolyzes only at high concentrations. In this respect, N-ethylmaleimide is like iodoacetate. There is a rapid and extensive reaction of N-ethylmaleimide

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>% Fall in GSH</th>
<th>% Hemolysis</th>
<th>% Fall in GSH</th>
<th>% Hemolysis</th>
</tr>
</thead>
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<td>5</td>
</tr>
<tr>
<td>6</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>31</td>
</tr>
</tbody>
</table>

with erythrocytic SH groups as shown by a decrease in absorption at 300 mμ, the reaction reaching completion within 60 min at pH 6.8, 25°, and 2 mM N-ethylmaleimide (Morell et al., 1959). About 1400 μmoles of N-ethylmaleimide react per 100 ml of erythrocytes, this being equivalent to approximately 3 moles of SH per mole of hemoglobin. There are about 2 μmoles of glutathione per millimeter of erythrocytes and N-ethylmaleimide react with these completely within 5 min, whereas p-MB cannot penetrate readily into the cells (Jacob and Jandl, 1962). Glucose utilization and lactate formation are readily inhibited by N-ethylmaleimide but not by p-MB. The facilitated transport of glucose is progressively depressed by N-ethylmaleimide at concentrations from 3.9 mM to 12.1 mM, but in contrast to the reaction with glutathione the rate of inactivation of the glucose carrier is slow, maximal inhibitions being reached only after several hours (Dawson and Widdas, 1963). The kinetics can be formally represented if there are assumed to be three types of SH group or carrier — 70% of a faster reacting component, 25% of a slower reacting component, and 5% of an unreactive component. The reaction has the fairly high activation energy of 27 kcal/mole (Q10 around 4) and the rate increases with rise in the pH from 5.5 to 8.5. Increase of the glucose concentration has no effect on the inhibition until it is greater than 4 mM and then the rate of reaction with the carrier is increased. That this is a selective effect on a glucose carrier is indicated by the complete lack of effect on malonamide penetration.

N-Ethylmaleimide causes a loss of K+ from duck erythrocytes but, in contrast to other inhibitors, also causes some loss of Na+, this being accompanied by marked suppression of glucose uptake and glycolysis (Tosteson and Johnson, 1957). There is also loss of water from the cells. K+ loss from human erythrocytes also occurs when exposed to N-ethylmalei-
mide 1–20 mM, and this is claimed to be mainly due to an increase in permeability to K⁺ (Giebel and Passow, 1961). This may be true at the beginning, when K⁺ loss is faster than Na⁺ uptake, but later may more likely be attributed to an inhibition of the active accumulation of K⁺. Jacob and Jandl (1962) found that K⁺ loss occurs when all the erythrocytic glutathione is reacted and when glycolysis starts to be inhibited, whereas p-MB causes early K⁺ loss unassociated with metabolic changes and presumably acts directly on the membrane. The ATP required for Na⁺—K⁺ transport is about half contributed from glycolysis, and 1 mM \(N\)-ethylmaleimide depresses this and shifts the source of energy so that, at least under certain conditions, the transport is not immediately affected (Sen and Post, 1964). The EM pathway, however, is not markedly inhibited since there is an elevation of pyruvate which partially compensates for the decrease in lactate. There is thus evidence both for and against the glycolytic inhibition being responsible for the loss of K⁺, but whether this is related to the disappearance of some of the glutathione is not known.

Miscellaneous Cell Functions

\(N\)-Ethylmaleimide like several other SH reagents slightly stimulates the motility of the rat intestine, this occurring maximally around 0.0057 mM, and blocks the stimulation produced by acetylcholine at 0.57 mM, at which time 76% of the SH groups have been blocked (Goodman and Hiatt 1964). Even at 0.001 mM it depresses the acetylcholine response, suggesting that the acetylcholine receptor may contain SH groups. It is not known if \(N\)-ethylmaleimide can release histamine, but at 1 mM it is able to completely abolish the histamine release induced by antigen in sensitized guinea pig lung (Edman et al., 1964). The reaction is not with the antigen or antibody so that some essential tissue component must be inactivated. We have seen that \(N\)-ethylmaleimide blocks the uptake of glucose in several tissues and this could play a role in the effects on muscle activity. Diaphragm muscle possesses a transport system for D-xylose and related sugars, and this is inhibited 80% by 1 mM \(N\)-ethylmaleimide (Battaglia and Randle, 1959, 1960). It was postulated that the carrier contains SH groups with which the sugars may combine, but inhibition by SH reagents could certainly be interpreted in several other ways. The transfer of phosphate across the membranes of staphylococci is an exchange diffusion process involving a carrier. \(N\)-Ethylmaleimide inhibits this exchange at pH 7 and it was concluded that the carrier possesses SH groups of rather low reactivity (Mitchell 1954). Again, there is no real evidence that the carrier itself contains SH groups.

The only investigation of \(N\)-ethylmaleimide on the heart gave the surprising result that it exerts a positive inotropic action on the cat papillary muscle (Bennett et al., 1958). Although several metabolic inhibitors
can augment contractility under certain circumstances, \(N\)-ethylmaleimide is the only SH reagent with this property. Furthermore, it is quite potent, acting at 0.1 mM. Only hypodynamic myocardium is stimulated. The R—CH=CH—CO—R' grouping was thought to be responsible for this action. \(N\)-Ethylmaleimide at 0.1 mM lowers the electrical potential across frog skin and from the changes in resistance it was suggested that, as with the mercurials, the reactive SH groups lie within the anion-transporting pores, the inhibitors acting sterically to impede ion flow (Janacek, 1962).

**EFFECTS ON MITOSIS AND GROWTH**

The initial work on \(N\)-ethylmaleimide was prompted by the attempt to find mitotic inhibitors related to maleate, and this substance was found to be one of the most potent of all the imides tested (Friedmann et al., 1949). Mitosis in chick fibroblast cultures is reduced 18\% by 0.0004 mM \(N\)-ethylmaleimide and the phase distribution is appreciably shifted, indicating a metaphase inhibitor. The effects increase with concentration up to 0.002 mM and then remain constant. No significant cytological abnormalities were observed. When \(N\)-ethylmaleimide is allowed to react with excess glutathione the antimitotic activity is mainly lost, but there is some residual effect that was thought might be due to the adduct itself (Friedmann et al., 1952b). The whole problem of the mechanism involved here remains to be solved and it is by no means certain that SH group reaction is the essential factor. Although it was stated that succinimide is inactive (Friedmann et al., 1949), it increases the number of cells in metaphase very significantly (from 27\% to 41\%), although mitosis in not greatly depressed. Furthermore, the very high potency indicates some very specific action; indeed, it is surprising that there is enough \(N\)-ethylmaleimide to produce an effect since at concentrations around 0.001 mM one would expect a large fraction to be reacted with nonfunctional components. In fact, the medium contained 0.15 mM glutathione, which would be expected to inactivate most of the \(N\)-ethylmaleimide before it had the opportunity to enter the cell (Friedmann, 1952). It is also surprising that no further work has been done on this interesting antimitotic effect.

\(N\)-Ethylmaleimide is potently bacteriostatic, inhibiting growth of *Staphylococcus aureus* and *Escherichia coli* 50\% at 0.025 and 0.016 mM, respectively (Marrian et al., 1953). Terminal division of *Erwinia*, one of the Enterobacteriaceae, triggered with Ca\(^{++}\) or pantoate is completely inhibited by 3 mM \(N\)-ethylmaleimide (Grula and Grula, 1962). Yeast, on the other hand, is much more resistant, 50\% inhibition of growth being produced by 8 mM \(N\)-ethylmaleimide (Loveless et al., 1954). The sensitivity of bacteria to X-radiation and \(\gamma\)-radiation is markedly increased by \(N\)-ethylmaleimide (Bridges, 1960, 1961). It was stated that 1 mM \(N\)-ethylmaleimide
is nontoxic to *S. aureus*, *E. coli*, and *Pseudomonas* sp., but when applied a few minutes before irradiation it increases the lethal effect both aerobically and anaerobically. Addition of *N*-ethylmaleimide after the irradiation does not alter the result. Bridges thinks that the inhibitor and the radiation must act simultaneously, and postulates that *N*-ethylmaleimide may react with some short-lived molecule or intermediate produced by the radiation, or with SH groups formed by the splitting of disulfide bonds by the radiation. Lynch and Howard-Flanders (1962) confirmed this effect in *Shigella sonnei* for 0.2 mM *N*-ethylmaleimide and found that the SH content of the cells is reduced around 75% within 30 min. It would be interesting to know if *N*-ethylmaleimide alters the response of simpler systems to irradiation. Vaccinia virus is quite readily inactivated by 0.1 mM *N*-ethylmaleimide and, as with the mercurials and iodoacetamide, the action is not to prevent attachment to the host cell but the release of nucleic acids within the cells (Allison, 1962). It may be noted that *N*-butylmaleimide has been introduced commercially as a disinfectant.

Plant growth can be inhibited by *N*-ethylmaleimide and van Overbeck *et al.* (1955) considered it to be an auxin antagonist, while Niedergang-Kamien and Leopold (1957) showed that at 0.01–1 mM it reduces auxin transport. Microspore mitosis in cultured lily anthers is inhibited by 2 mM *N*-ethylmaleimide and since there is a 30% decrease in thiol content it was assumed that the inhibition is attributable to this, resumption of mitosis after 4–5 days being due to the biosynthesis of new thiols (Stern, 1959). Nothing is known of the inhibiting effects of *N*-ethylmaleimide on tumor cells, but Belkin and Hardy (1961) observed that at 0.1 mM there is blebbing of Sarcoma 37 ascites cells, this usually being of the scalloped type, although in other tumor cells different blebbing patterns were seen. Such a blebbing reaction also occurs *in vivo* following the injection of *N*-ethylmaleimide. Blebbing is generally considered to be a reaction brought about in the membranes as a result of SH group reaction, this altering the membrane structure and water permeability.
CHAPTER 4

ALLOXAN

Alloxan is an unstable SH reagent which reacts readily with certain thiols but not with most protein or enzyme SH groups. It is thus not a reliable SH titrator but exerts inhibitions of interest and, like iodoacetate and \( \text{-ethylmaleimide} \), is a valuable tool because of its selectivity. The modern interest in alloxan stems from the observation of Dunn and his associates (1943 a) in Glasgow that it can produce specific pancreatic islet necrosis in rabbits. This had been observed in their laboratories as far back as 1926, in studying renal lesions induced by urate and related compounds, but had not been published. Jacobs (1937) had indeed reported that alloxan exerts profound effects on blood glucose levels, but had observed only the early hypoglycemia and did not study the animals sufficiently long to note the development of the later diabetic hyperglycemia. Dunn and his group also did not observe the animals long enough to characterize the diabetes produced as the result of islet destruction, but the occurrence of typical diabetes was reported by Brunschwig et al. (1943) and Goldner and Gomori (1943) in Chicago, and a month later independently by Bailey and Bailey (1943) in Boston. It was by now clear that damage to the islet tissue is confined to the insulin-secreting \( \beta \)-cells, the \( \alpha \)-cells being resistant to alloxan, and this differential action was demonstrated particularly well by Dunn et al. (1944). These results provided an easy and specific method for inducing experimental diabetes in animals and this has contributed greatly to the study of diabetes. In addition, it has posed the very fascinating problems of the selective action of alloxan on a single type of cell and the mechanism by which alloxan initiates within a few minutes the progressive destruction of these cells. On the tissue level alloxan is one of the most selective inhibitors known.

At the time of the discovery of its diabetogenic action, alloxan had been known for over a century, but only sporadic reports on its biological effects had appeared. It was synthesized by Wöhler (1838), its reactions with amino acids were studied by Streeker (1862), the possibility of its natural occurrence in animals was indicated by von Liebig (1962), Lang (1866) and Ascoli and Izar (1909), and its reactions with SH groups were
reported by Wieland and Bergel (1924), Labes and Freisburger (1930), and Lieben and Edel (1932). Inhibitions of several enzymes had been found by various workers, including Purr and Weil (1934), Hopkins et al. (1938), Bernheim (1938), and Lehmann (1939). Lusini (1894) and Koehme (1894) had shown that alloxan is rapidly and completely metabolized in the body, and others had provided evidence that it disappears rapidly from neutral or alkaline solutions. Alloxan was thus known in 1943 to be a very unstable substance capable of oxidizing SH groups and exerting effects on various enzymes and metabolic systems, but there was no clue to the mechanism involved in the β-cell necrosis. One must be careful to distinguish between the direct effects of alloxan in animals and the secondary effects resulting from the alterations of the β-cells and insulin release. There have been many studies on animals made diabetic by the administration of alloxan, but such work is not within our province inasmuch as these secondary effects are related only to insulin deficiency and are typical of the diabetic state rather than the immediate actions of alloxan. The following reviews on the diabetogenic action of alloxan are recommended: Duff (1945), Goldner (1945), Lukens (1948), Houssay (1950), Lazarow (1954a), and Falkmer (1961).

**CHEMICAL PROPERTIES**

The structure and reactions of alloxan in aqueous solution are poorly known because relatively little work has been done on its chemistry since the modern concepts of chemical structure and reactivity were formulated, and also because it is a complex, unstable, and reactive substance. Even its reactions with thiols have not been adequately investigated and for this reason it is difficult to understand the effects on enzymes and particularly how it can selectively damage the pancreatic β-cells. Although alloxan is a tetraoxypyrimidine, its properties differ in several respects from the usual pyrimidines, due probably to the presence in alloxan of the —CO—CO—CO—grouping, this supposedly containing a highly reactive central CO group.

**Acid-Base Properties**

The dioxypyrimidines, such as uracil and thymine, are very weak acids (pK$_{a1}$ = 9.5) but alloxan is definitely stronger and probably exists at physiological pH mainly in the anionic form. The pK$_{a3}$ of alloxan was given as 7.20 by Richardson and Cannan (1929) and as 6.63 by Labes and Freisburger (1930). The acidity is due to the lability of the H atoms resulting from enolization and to the increased possibilities for resonance following ionization. The ionization is usually represented as:
but the location of the negative charge and the remaining H atom is indefinite (see following section). The dissociation of the second proton does not occur readily and \( pK_a \) is around 10 or above (Richardson and Cannan, 1929).

**Structure**

Alloxan in the solid state can exist in the anhydrous form or as the monohydrate or a polyhydrate, all crystalline. It has usually been assumed that alloxan in solution is the monohydrate (Brückmann and Wertheimer, 1947; Patterson et al., 1949 a; Resnik and Wolff, 1956), as a result of the reactivity of the 5-CO group, but the dihydrate in aqueous solution has also been suggested (Lagercrantz and Yhland, 1963). Although this may be true, there is little or no evidence for either such a hydrated form in solution or for the localization of the water at the 5-CO group. If hydration of this group arises because of adjacent CO groups, it would seem that enolization at the 4- and 6-positions would reduce the reactivity of the 5-CO group and the likelihood of hydration. This problem is probably not critical and most of the important reactions of alloxan can be written with either form.

The question of the keto-enol tautomerism is more important but cannot be answered satisfactorily in the present state of our knowledge. There have been different opinions as to the keto-enol equilibria in the commonly occurring hydroxypyrimidines, but recently more workers have favored the dominance of the keto form. The C—O bond distance in the crystalline state is 1.25 Å, which is much closer to the C=O bond than the phenolic bond; however, in aqueous solution the situation may be different. Comparison of the ultraviolet absorption spectra of pyrimidines with the corresponding \( N \)-substituted compounds indicates the keto form to be predominant, and it was concluded that the 2-, 4-, and 6-OH pyrimidines
should be represented mainly in the keto form. Nevertheless, the C—C and C—N bond lengths indicate at least 50% double bond character, so some aromatic resonance must occur. In any event, even though the pyrimidines may generally be in the keto form, alloxan presents additional problems, and the lower pK\textsubscript{a1} points to more extensive enolization in alloxan. Alloxan has two H atoms and they are probably not localized but migrate between the N and O atoms. There is a total of 8 tautomeric forms for alloxan: 1 all-keto form, 4 monohydroxy forms, and 3 dihydroxy forms. The relative importance of these forms remains to be determined.

Ionization of alloxan makes it more difficult to distinguish between keto and enol forms since the anion can resonate between the structures:

\[
\begin{align*}
\text{O} & \quad \text{O}^- \\
\text{C—N—} & \quad \text{C—N—}
\end{align*}
\]

There are thus 16 structures which can be written for the alloxan anion, there being 5 resonance hybrids. Inasmuch as alloxan is bilaterally symmetrical and it is unlikely that any one structure is markedly dominant, it might be better to represent the alloxan anion as:

providing the δ-charges are not assumed to be equal. The negativity is probably more associated with the O atoms than the N atoms. Although the anion is definitely more unstable than the neutral molecule, which species is more important for enzyme inhibition or β-cell damage is not known.

### Oxidation-Reduction Reactions

Alloxan is readily reduced to dialurate\(^*\) and this was studied in detail by Richardson and Cannan (1929), who determined the values shown in the following tabulation for \(E'_0\):

<table>
<thead>
<tr>
<th>pH</th>
<th>(E'_0) (mv)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.56</td>
<td>+ 78.9</td>
</tr>
<tr>
<td>7.03</td>
<td>+ 60.2</td>
</tr>
<tr>
<td>7.50</td>
<td>+ 36.0</td>
</tr>
</tbody>
</table>

\(^*\) Dialuric acid was found to have a pK\textsubscript{a1} of 2.83 by Richardson and Cannan (1929), so it presumably exists at physiological pH as the dialurate ion; no other ionizing groups were detected. This pK\textsubscript{a1} is surprisingly low for the structure depicted for dialuric acid.
$E_0$ is 364 mv. The reduction reaction is usually written as:

\[
\text{Alloxan} + 2 \text{H} \rightleftharpoons \text{Dialuric acid}
\]

or as a removal of an O atom from the monohydrate. Dialurate is oxidized to alloxan by $O_2$ and many other oxidants, so that it is diabetogenic by virtue of its conversion to alloxan.

It is somewhat surprising that the quinonoid structure for alloxan, which one would expect to be of some importance, is not reduced to isodialuric acid, the aromatic resonance stabilizing the tetrahydroxy structure. This is an important point with regard to the reaction with thiols and the formation of alloxantin. It is questionable if the characterization of these substances is as precise or reliable as is usually assumed, and perhaps more attention should be paid to the quinonoid character of alloxan.

Reduction of alloxan is often accompanied by the formation and precipitation of the relatively insoluble alloxantin, which is usually formulated as shown, arising by conjugation of alloxan and dialuric acid, but again one can speculate that alloxantin is a molecular compound such as is formed from quinones and hydroquinones (i.e., a quinhydrone), possibly by hydrogen bonding. This might be expected to occur with alloxan and isodialuric acid. The rates of formation and splitting of alloxantin appear to be very fast. The equilibrium constant, $K = (\text{alloxan}) \ (\text{dialuric acid})/(\text{alloxantin})$,
was found by Richardson and Cannan (1929) to be 0.048 at a pH of 1.08, but unfortunately it was not possible to determine the value near neutrality.* Electron-spin resonance studies show that free radicals are formed during the reduction of alloxan by glutathione, and these free radicals may play a role in the formation of alloxantin (Lagercrantz and Yhland, 1963).

Stability of Alloxan

Crystalline alloxan is not stable at room temperature in air, and decomposes slowly to form alloxantin, oxalate, urea, and CO₂ (and possibly alloxanic acid) (Archibald, 1945). Several impurities may be present in commercial samples of alloxan. Pure alloxan does not give a purple color with Ba(OH)₂ but several of the decomposition products do, so that this may be used as a rough test of purity. In accurate work it is advisable to prepare alloxan (Hartman and Sheppard, 1955), obtain a fresh preparation from commercial sources, or recrystallize it in the anhydrous form from acetone or glacial acetic acid. It should be stored near 0° in the dark. Since alloxan is reasonably stable below pH 3.5, acid solutions may be made up and neutralized immediately before use, or in animal work injected without neutralization.

The instability of alloxan in neutral solutions has been long recognized, and was noted by Dunn et al. (1943 b) in their initial studies on the diabetogenic action, neutralized solutions rapidly becoming inactive. Karrer et al. (1945) found that a 7 mM alloxan solution kept at pH 7.5–8 and 37° contained no alloxan after 15 min, and Lazarow et al. (1948) stated the half-life of alloxan in solution at pH 7.4 and 37° to be about 1 min. In solutions containing biological material, such as serum, blood, or tissue extracts, alloxan is even more unstable due to other reactions not occurring in simple solutions (Leech and Bailey, 1945) and disappearance of alloxan may take place within a few seconds, even at a lower temperature (Brückmann, 1946). The effects of blood components are well seen in the results of Veksler (1956), but the rates of destruction are relatively slow because of the low pH (Fig. 4-1).

The instability is due to the conversion of alloxan to alloxanate by an internal benzilic acid rearrangement (Schlieper, 1845). The rate of the conversion was stated by Labes and Freisburger (1930) to be proportional to the concentration of the alloxan anion, so that the rearrangement would depend on enolization as in the scheme outlined by Klebanoff and Greenbaum (1954). However, Resnick and Wolff (1956) found that N, N'-dimeth-

* I regret being so perverse as to be critical of the customary formulations of these compounds and their reactions, but after surveying the early literature on the structural characterizations of alloxan derivatives, I am not convinced that they are correct.
yalloxan decomposes even more rapidly than alloxan; since enolization cannot occur in this compound it was concluded that enolization with subsequent ionization is not important, and the reaction was written as base-catalyzed. It was always assumed that C-4 joined to C-6 in the rearrangement, the carboxylate group arising from C-5, but recent work has cast doubt on this (Kwart and Sarasohn, 1961; Kwart et al., 1961).

When alloxan-5-C\textsuperscript{14} is converted to alloxanate, the labeling is found not in the carboxylate group but the ring, so there is not only a C—C shift but also a C—N shift involved, C-5 joining to N-1. It was also shown that the dianion undergoes a similar rearrangement, so that the dependence on

![Reaction diagram](image)

**Fig. 4-1.** Destruction of alloxan at pH 5.5-6.2 and 16°C: (1) whole blood; (2) plasma; (3) serum; (4) phosphate buffer. (From Veksler, 1956.)
pH is somewhat more complex than previously thought. One should avoid using borate buffers in work with alloxan since borate accelerates the conversion to alloxanate, possibly through a Boeseken type of ester intermediate (Seligson and Seligson, 1951 a).

The decomposition of alloxan is strongly dependent on the pH, increase in the pH from 6 to 8 markedly speeding the reaction, which was the basis for the assumption by Labes and Freisburger (1930) that the conversion depends on the concentration of the anion. The rate levels off above pH 7.5–8 and is no faster at pH 11 (Richardson and Cannan, 1929). The effect of pH is well seen in the results of Patterson et al. (1949 a) (Fig. 4-2), and similar curves were obtained by Seligson and Seligson (1951 b). Since alloxanate is not a potent oxidant, does not react with SH groups, and is nondiabetogenic, it is of the utmost importance in work with alloxan to minimize its inactivation before and during experiments. It is clear that both in enzyme preparations and in the blood of injected animals the alloxan must be lost rapidly, not only by conversion to alloxanate but by reduction to dialurate. Furthermore, the conversion of alloxan to alloxanate is usually accompanied by a fall in the pH, since alloxanic acid is a much stronger acid than alloxan. Archibald (1945) noted that a 7 mM solution of alloxan

![Fig. 4-2. Destruction of alloxan at 37° in solutions at different pH's. (From Patterson et al., 1949 a.)](image)

...
quickly acquires a pH of 3.1, which stabilizes the alloxan which has not been converted. This marked fall in pH must be considered in \textit{in vitro} studies and it is possible that some of the inhibitions of enzymes reported for alloxan may have been due to a pH effect. It may also be pointed out that the search for more stable alloxan derivatives has failed: the \(N\)-substituted derivatives decompose more rapidly than alloxan (Brückmann and Isaacs, 1949), and alteration of the rest of the molecule abolishes the ability to react with SH groups and the diabetogenicity.

**Reactions with Amino Acids**

Strecker (1862) demonstrated a reaction between alloxan and certain amino acids yielding \(\text{CO}_2, \text{NH}_4^+\), and an aldehyde with one less C atom than the amino acid. Alloxan is transformed into purpuric acid, and this may complex with the amino acids to give red or rose-colored products; indeed, the glycine complex has been isolated (Piloty, 1909). Hurtley and Wootton (1911) showed that most amino acids except glycine and proline react in this way. A mixture of alloxan and glycine does not form formaldehyde in good yield; the reaction is quite complex and the alloxan is reduced to dialurate and hence to alloxantin, this reacting with the ammonia produced to give purpuric acid or murexide. Lieben and Edel (1932) gave the following order of decreasing reactivity with alloxan: cysteine, histidine, glycine, glutamate, tryptophan, phenylalanine, arginine, aspartate, tyrosine, leucine, serine, alanine, valine, and lysine. The presence of a free amino group is necessary but not sufficient, since many amines do not react, and no satisfactory rule for reactivity could be formulated. Abderhalden (1938) isolated some of the aldehydes formed in this reaction and showed that certain polypeptides react to give a rose or blue-violet color if a terminal glycine is present. The more recent study of Schönberg \textit{et al.} (1948) showed the necessity for the \(-\text{CO}-(\text{CH}=\text{CH})_n-\text{CO}¬\) grouping and the following reaction scheme was advanced:

\[
\begin{align*}
\text{R} & \quad \text{R} \\
\text{R} & \quad \text{R} \\
\text{C}=\text{O} & \quad \text{C}=\text{O} \\
\text{H}_2\text{N}-\text{CH}-\text{COO}^- & \quad \text{C}=\text{N}-\text{CH}-\text{COO}^- \\
\text{C}=\text{O} & \quad \text{C}=\text{O} \\
\rightarrow & \quad \text{C}=\text{N}-\text{CH}_2-\text{R} \\
\rightarrow & \quad \text{C}=\text{N}-\text{CH}-\text{R} \\
\rightarrow & \quad \text{C}=\text{OH} \\
\rightarrow & \quad \text{C}=\text{OH} + \text{R}-\text{CHO} \\
\end{align*}
\]

Such reactions are now known as Strecker degradations and are analogous to the reactions of ninhydrin with amino acids. The red-purple color of the product arises from murexide, which is the ammonium salt of purpuric acid, and the complexes of the amino acids with these substances; thus the color produced by reaction of alloxan with biological materials is often intensified by the addition of \(\text{NH}_4^+\).
Reactions with Thiols

Alloxan can oxidize cysteine to cystine and GSH to GSSG, being simultaneously reduced to dialurate. The dialurate may complex with alloxan to form alloxantin or, under aerobic conditions, may be reoxidized to alloxan. Reduction of alloxan by \( \text{H}_2\text{S} \) yields dialurate when there is excess \( \text{H}_2\text{S} \) but alloxantin when the \( \text{H}_2\text{S} \) is nearly equimolar with the alloxan (Tipson and Cretcher, 1951). The reaction with cysteine anaerobically may be written as:

\[
2 \text{ Alloxan} + 2 \text{ cysteine} \rightarrow \text{ alloxantin} + \text{ cystine}
\]

It is interesting that Lieben and Edel (1932) found cysteine to be the most reactive amino acid in producing the red-purple color with alloxan. They concluded that the color reaction observed with proteins is due mainly to the SH groups, inasmuch as it parallels the nitroprusside reaction in various materials. Furthermore, urea-denatured ovalbumin reacts more readily than the native protein, although this does not prove participation of the SH groups. Actually, reduction of alloxan by SH groups alone would not yield a colored product (unless perhaps \( \text{NH}_4^+ \) is present) and it is thus doubtful if Lieben and Edel were measuring the reaction with SH groups.

The problem was made more complex, but also more interesting, by the finding of Lazarow et al. (1948) that the reaction between alloxan and glutathione gives rise to products with absorption maxima at 270 m\( \mu \) and 305 m\( \mu \); these peaks are not related to the same substance and can vary independently. The peak at 305 m\( \mu \) occurs when equimolar amounts of the reactants are mixed, even at concentrations as low as 0.2 mM. The rate of the reaction is rapid, being 75% complete in 3 min at pH 7.4 and 37°. Alloxan does not react with GSSG and dialurate does not react with GSH. Thus, in addition to the oxidation of SH groups, there is another reaction whereby a complex is formed. Complex formation also occurs with cysteine when it is present in an equimolar ratio with alloxan, but if cysteine is in excess, reduction of alloxan may be dominant (Patterson et al., 1949 b). The product with the peak at 305 m\( \mu \) was called Complex 305 and its structure was represented by Lazarow as:

\[
\begin{align*}
\text{HN} & \quad , \quad \text{OH} \\
\text{O} & \quad \text{N} \quad \text{OH} \\
\text{OH} & \quad \text{OH}
\end{align*}
\]

\[
\begin{align*}
\text{HS} & \quad \text{CH}_2 \\
\text{CH} & \quad \text{CO} \quad \text{glycine} \\
\text{HN} & \quad \text{glutamate}
\end{align*}
\]

\[
\begin{align*}
\text{HN} & \quad \text{OH} \\
\text{O} & \quad \text{N} \quad \text{CO} \\
\text{N} & \quad \text{glutamate}
\end{align*}
\]

Complex 305

this being analogous to the reaction of alloxan with \( o \)-phenylenediamine (see page 377). This structure was questioned by Resnick and Wolff (1956).
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because \( N, N' \)-dimethylalloxan yields a similar product with GSH, although the absorption at 305 m\( \mu \) is less; the complex is not identical with that from GSH, but the chromophore group must be essentially the same. They showed that the Strecker reaction is not involved. Alloxanate does not react with GSH to increase absorption at 305 m\( \mu \), but possibly the formation of Complex 305 involves a ring rearrangement. Alteration of the 5-CO group leads to loss of reactivity with SH groups. In searching for a grouping with the ability to absorb at 305 m\( \mu \), they noted that violuric acid has a peak at 312 m\( \mu \) and the reaction product of alloxan and \( o \)-phenylenediamine has a peak at 305 m\( \mu \). Both compounds contain the \(-N=C—C=O\) group. They suggested that Complex 305 might contain a thiazine ring, the pyrimidine ring of alloxan being opened. They pointed out that

\[
\text{H}_2\text{N}—\text{CONH—CO}^+ \text{glycine}
\]

there is no real evidence for this structure. It would be important to elucidate the nature of this reaction, inasmuch as it may have an important bearing on the inhibition of enzymes and metabolism, and perhaps the diabetogenic activity.

Alloxan is able to react with coenzyme A as determined by preincubation and measuring the activity of the coenzyme A with the liver acetylating enzyme (Cooperstein and Lazarow, 1954, 1958). Incubation of alloxan with coenzyme A for 1 hr at room temperature and pH 7.4 leads to a 15–30\% inactivation of the coenzyme A when the alloxan is 0.5 mM, but at 0.25 mM there is insignificant reaction. The reaction with coenzyme A may be a simple oxidation-reduction since cysteine is able to restore most of the activity.

**Reaction with \( o \)-Phenylenediamine**

It has frequently been assumed that alloxan and \( o \)-phenylenediamine react directly to form an isoalloxazine ring, or flavin-like derivative, but it has been suggested that a splitting of the pyrimidine ring occurs to give a quinoxaline ring (King and Clark-Lewis, 1951; Barlow et al., 1951).

\[
\text{H}_2\text{N}—\text{CONH—CO}
\]

This reaction is of some importance because it is used in certain determi-
nations of alloxan, may provide a model for the reaction with thiols, and presents the possibility that alloxan might react with appropriately spaced amino groups on proteins.

Chelation with Metal Ions

Early work indicated that alloxan forms complexes with Zn$^{++}$ (Deniges, 1903) and various metal ions, and we shall see that some theories of the diabetogenic action have been based on metal ion chelation. However, most of this work was done in neutral or alkaline solutions, in which alloxan is unstable, so it is questionable whether the complexes observed were with alloxan or with the products of its decomposition. Thus Dubský et al. (1940) showed that alloxan and Fe$^{++}$ do not react to form a blue complex, as previously thought, and that the reaction is actually with alloxantin. Resnick and Cecil (1956) also showed that the supposed reaction between alloxan and Cu$^{++}$ is actually a chelation of the Cu$^{++}$ by alloxanate formed from the alloxan. However, Lange and Foye (1958) claim that 1:1 complexes of alloxan with Co$^{++}$, Ni$^{++}$, and Cu$^{++}$ occur. They formed the complexes at pH 7.5 and decreased the pH to 5.6 to prevent conversion to alloxanate, obtaining colored precipitates. The chelates were represented by the following structure:

\[
\begin{align*}
\text{Me} & \quad \text{N} & \quad \text{O} \\
\text{O} & \quad \text{N} & \quad \text{O} \\
\text{O} & \quad \text{O} & \quad \text{O}
\end{align*}
\]

Although chelation is probably not involved in the diabetogenic action, it would be important to characterize the reactions of alloxan and its products with metal ions inasmuch as certain enzyme inhibitions may depend on this.

Miscellaneous Reactions

Alloxan can oxidize a variety of substances and, since dialurate is autoxidizable, this occasionally leads to an uptake of O$_2$. Thus a rapid consumption of O$_2$ occurs when alloxan and ascorbate are mixed (Margules and Griffiths, 1950). Incubation of equimolar mixtures of alloxan and ascorbate at 30° leads to the rapid disappearance of the ascorbate, 40% being gone in 2 min and all by 30 min (Nath and Bhattachirty, 1955). Alloxan reacts quite readily with cyanide to form oxalurate but the cyanide acts only as a catalyst for a simultaneous oxidation and reduction of alloxan, dialurate being formed (Archibald, 1945). Hence, one must be careful in using alloxan and cyanide together. Alloxan also reacts with testosterone, progesterone, and other steroids, but less so than do uracil and various py-
rimidines (Molinari and Lata, 1962). The interactions apparently are so weak that it is doubtful if they contribute at all to the metabolic actions of alloxan.

Reactions with Proteins

The following reactions of alloxan with proteins might be postulated to occur: (1) oxidation of protein SH groups with reduction of alloxan to dialurate; (2) a Strecker reaction with terminal amino groups leading to the formation of purpuric acid or murexide; (3) reaction with two amino groups analogous to those in o-phenylenediamine to form a quinoxaline structure; (4) formation of a compound related to Complex 305 by reaction with properly spaced SH and amino groups; (5) association with the protein by hydrogen bonding, since there is ample opportunity for such bonding in alloxan; (6) chelation by alloxan, or by alloxanate formed from it, of metal ions associated with the protein. In none of the few investigated instances is the reaction with protein understood. Denatured ovalbumin was claimed to exhibit an increased absorption at 305 m\(\mu\) when treated with alloxan (Lazarow et al., 1948), but native crystalline ovalbumin is more resistant, although some reaction takes place (Patterson et al., 1949 b). Resnick and Wolff (1956) could detect no reaction with native ovalbumin, and found only a plateau between 285 and 305 m\(\mu\) to appear when denatured ovalbumin reacts with alloxan. The evidence for a reaction like that with GSH is thus equivocal. Brada (1949) reported that alloxan-treated ovalbumin is more resistant to trypsin digestion, but the reaction of the alloxan with the ovalbumin was carried out over 24 hr and with 2–10 mM alloxan, so that it is doubtful if a reaction of this type could be important biologically. Some proteins, such as scarlet fever toxin, appear to be completely resistant to alloxan (Barron et al., 1941). Alloxan inactivates complement \textit{in vitro} but this was found to be due entirely to the fall in pH, reactivity being restored following neutralization (Ecker et al., 1939).

Determination of Alloxan

There are numerous quantitative methods for the determination of alloxan, some of them applicable to biological material. Archibald (1945) reviewed the methods and listed six of value. The fluorometric determination is the most sensitive (0.02–0.2 \(\mu\)g) but is not very specific; colorimetric tests are less sensitive (0.02–0.2 mg) but more specific; gasometric and titrimetric methods are least sensitive (2 mg) but quite accurate. Leech and Bailey (1945) used both ferricyanide and tungstate in colorimetric tests for alloxan in the blood, but these are neither very sensitive nor specific. The formation of a blue color upon reaction of Fe\(^{++}\) with alloxantin, which is appropriately formed from alloxan, is very specific and reasonably sensitive (0.04–0.4 mg) (Brückmann, 1946; Dubsky et al., 1940) and viol-
urate formed from alloxan may be similarly detected. The fluorometric methods devised by Karrer et al. (1945) and Tipson and Cretcher (1950) are to be preferred, especially the latter. In the former case, alloxan was reacted with N-methyl-o-phenylenediamine to form a fluorescent derivative, thought to be 9-methylisalloxazine but probably not, and, in the latter case, riboflavin is formed by condensation of alloxan with D-1-ribitylamino-2-amino-4,5-dimethylbenzene, this method being able to measure alloxan concentrations down to 0.01 µg/ml or 0.00007 m.M. It might be suggested that in determinations of alloxan in biological material, especially in attempts to detect endogenous alloxan, several methods be used to increase the specificity.

**DIABETOGENIC ACTION**

We shall depart from the usual sequence of presentation in these volumes in the discussion of alloxan inasmuch as the production of pancreatic islet damage and diabetes is the most interesting aspect of this inhibitor, and for this reason it may be best to summarize briefly what is known of this before discussing the effects of alloxan on enzymes and metabolic pathways. Alloxan in the proper dosage can specifically damage the pancreatic islet $\beta$-cells, resulting in their disappearance within a few days, accompanied by a typical and permanent diabetes mellitus due to the failure in insulin secretion. If the dosage of alloxan is higher than required to damage the $\beta$-cells, definite effects may be observed on other tissues, especially the liver and kidney, and these effects, although not involved in the production of diabetes, are of interest in connection with the mechanisms of alloxan action.

**Description of Islet Changes**

The cytological changes observed are reasonably uniform in many species. The following summary is taken mainly from Dunn et al. (1943 b), Lukens (1948), and Lazarus et al. (1962). The rapidity with which visible changes in the $\beta$-cells can be induced by alloxan injections is remarkable. Within 5 min there is a diminution of cytoplasmic and nuclear granules, the cytoplasm becoming more homogeneous or foamy, and this is accompanied by a shrinkage of the cells. These changes proceed and by 15–30 min the cytoplasm is clearer, the cells more shrunken, and the nuclei are slightly pycnotic. Tiny vacuoles can now be observed in the cytoplasm and these grow and coalesce until the cells are mainly vacuolated. Nuclear pycnosis also progresses with chromatin clumping and eventual disintegration of the nuclear membrane between 5 and 10 hr after administration of the alloxan. By 24 hr the $\beta$-cells are mainly destroyed and the islets contain cellular debris and within 3–5 days no $\beta$-cells may be visible, although the
α-cells and the acinar tissue are normal. Usually there is no accompanying inflammatory reaction, fibrosis, or hyalinization. The general picture is one of a selective progressive necrosis of the β-cells leading to their complete obliteration. The mitotic phase distribution of islet cells is shifted, showing a retardation of metaphases and an increase in anaphases (Faller, 1953). Chromosomes may be clumped on the equatorial plate and chromatin bridges are visible. Electron microscopy studies show essentially the same changes but the early vacuolization is even more apparent, especially in the mitochondria, and evidences of karyolysis are seen at an earlier stage (Lacy and Cardeza, 1958; Williamson and Lacy, 1958). In certain fish, e.g. the sculpin (Cottus scorpius), there is segregation of the β-cells making them more easily visualized, and here similar ultrastructural changes have been observed (Falkmer and Olsson, 1962). It is very interesting that rats given several small daily doses of alloxan exhibit islet dysfunction without observable changes in the β-cells (Molander and Kirschbaum, 1949). The animals are not made totally diabetic by this procedure but it demonstrates that functional disturbance can be induced without gross cell damage.

**Alterations of Blood Glucose**

Administration of alloxan leads to a characteristic triphasic variation of the blood glucose in mammals, birds, reptiles, and certain fish. There is a rapid initial hyperglycemia lasting for 3–4 hr, followed by a temporary hypoglycemia usually of 6–12 hr duration, and finally a rise to a permanent diabetic hyperglycemia. The exact time relations vary with the species and to some extent the dose. There is an unexplainable variation in response among individuals and a certain fraction in a population seems to be quite resistant. The degree of the initial hyperglycemia can be correlated with the eventual appearance of the diabetic state, and thus it was concluded that the final fate of the animal is determined within a few minutes of the injection (Lisewski and Mohnike, 1959 b). The hypoglycemia, however, is greater in those animals showing a small initial hyperglycemia and failure to develop diabetes. A typical blood glucose curve is shown in Fig. 4-3. Gaarenstroom and Siderius (1954) believe there is initially both a hypo- and a hyperglycemic effect of alloxan, the latter usually masking the former.

Early death from alloxan is usually due to the severe hypoglycemia which is often produced. Jacobs (1937) was the first to show that convulsions during this period can be readily prevented by the administration of glucose, and it is now common practice in the production of alloxan diabetes to protect the animals during the first day by injecting glucose as needed, since this procedure does not affect the development of diabetes, as shown by Bailey and Bailey (1943).

An unexpected response to alloxan has recently been observed in hereditarily obese hyperglycemic mice (Solomon and Mayer, 1962 a, b). These
mice are characterized by islet hyperplasia and high pancreatic insulin levels, hyperinsulinemia, and abnormal adipose tissue metabolism. Instead of producing a sustained rise in blood glucose, alloxan causes a marked fall from the initially high levels (see accompanying tabulation). No ex-

<table>
<thead>
<tr>
<th>Blood glucose (mg%)</th>
<th>Normal</th>
<th>Obese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initially</td>
<td>159</td>
<td>347</td>
</tr>
<tr>
<td>After 3 days</td>
<td>428</td>
<td>152</td>
</tr>
<tr>
<td>After 28 days</td>
<td>427</td>
<td>233</td>
</tr>
<tr>
<td>After 56 days</td>
<td>455</td>
<td>257</td>
</tr>
</tbody>
</table>

planation for this paradoxical effect could be given but it may be an important observation which could help in solving the problem of the mechanism by which alloxan acts on the β-cells.

![Blood glucose changes in rabbits after intravenous injection of 200 mg/kg alloxan. (From Goldner and Gomori, 1944.)](image)

During the diabetic hyperglycemic phase the blood levels of pyruvate, α-ketoglutarate, acetone, and acetoacetate are markedly elevated in rats
given alloxan, just as they are in diabetes of any other origin, and these changes are undoubtedly due to the hypoinsulinemia rather than to a direct effect of the alloxan (El Hawary, 1955; El Hawary and Thompson, 1954). The hyperlipemia and hypercholesterolemia seen around 5–10 days after the administration of alloxan to rabbits are also caused mainly by the diabetic state, since the levels can be reduced by insulin (Kendall et al., 1945). However, certain other blood changes may be due to the alloxan directly. Thus alloxan causes marked uricacidemia 12–36 hr after injection in pigeons (Saviano and Leone, 1946) and a creatinuria, accompanied by increased K+ and decreased phosphate excretion, in rabbits (Bruns and Wüst, 1952). Whether these changes reflect an action of alloxan on the kidney or on tissue metabolism is not known.

Diabetogenic Doses and the Factors Determining Susceptibility to Alloxan

The effectiveness of alloxan in producing selective β-cell destruction and permanent diabetes in a particular species depends on several factors. One of these factors must be the blood thiol level: the greater the thiol concentration, the less alloxan reaches the pancreas. Swenson et al. (1959) found that various strains of mice respond differently to alloxan, and that the more resistant strains have higher erythrocytic GSH, so that both GSH levels and alloxan susceptibility seem to be genetically controlled. Another factor is the blood glucose concentration (see page 390) and this may be altered by the diet or other means. Saito et al. (1959) showed that the diabetic response in rats is most frequent when the animals have been on a high protein, high fat, and high calory diet. It has become more and more evident that, to achieve the most consistent results, it is necessary to starve the animals 24–60 hr before the administration of alloxan. Kass and Waisbren (1945) developed a reliable method for producing diabetic rats by starving the animals for at least 48 hr prior to the injection of alloxan, in which case 175 mg/kg of alloxan gives 90–95% chronically diabetic animals when administered subcutaneously. If the rats are not starved, only 25% become diabetic. A third factor is apparently the age of the animal. Lisewski and Mohnike (1959 a) attempted to correlate the diabetic response in rabbits with various factors, such as body weight, plasma protein, and other variables, but the only statistically valid correlation was with age, younger animals being less sensitive to alloxan. A fourth important factor is the rate at which the alloxan is injected intravenously, as would be expected of a substance which is very unstable. Pincus et al. (1954) established that lower doses (around 75 mg/kg) are effective in rabbits when injected within 1 min but completely ineffective if over 5 min are taken; higher doses can be injected more slowly. As alloxan is injected into the blood stream, a certain fraction is destroyed in the blood and another fraction is removed by the tissues; if the rate of
injection is too slow, insignificant amounts of alloxan may reach the islet tissue. For this reason, the site of the injection may also be important. Also related to this factor is the fact that divided doses given at intervals are often less effective than single doses (Meade and Klitgaard, 1960). A single subcutaneous dose of 200 mg/kg to rats produces diabetes; if this is divided into four 50 mg/kg doses given every 2 hr, the effect is less; if divided into eight 25 mg/kg doses given every hour or every 2 hr, it is ineffective.

Some species such as chickens, ducks, carnivorous birds, and toads are relatively or completely resistant to alloxan with respect to the production of chronic diabetes. Among mammals there is some variation in susceptibility: guinea pigs (Maske and Weinges, 1957) and monkeys (Ramfjord, 1952) can be made diabetic only with difficulty and relatively high toxic doses. Perhaps \(\beta\)-cell destruction can be produced in all mammals, but in some the doses required are such that other tissues are damaged. It is interesting that dogs can be made resistant to alloxan by administering small repeated doses over several days, at which time they can receive 150 mg/kg intravenously, which is 3 times the diabetic dose in normal animals (Loubatières, 1948).

The diabetogenic dose was defined by Goldner (1945) as that which will produce in 80\% of the animals a sustained diabetic hyperglycemia without observable effects on other tissues. Although other workers have often not specified comparable doses, the intravenous diabetogenic doses in various species may be given as: rat 45–50 mg/kg (Brückmann and Wertheimer, 1947; Kipnis and Cori, 1957), dog 50–75 mg/kg (Goldner and Gomori, 1943), hamster 60 mg/kg (Harris et al., 1946), sheep 75 mg/kg (McCandless et al., 1948), mouse 100 mg/kg (Tocus and Cavallo, 1961), cat 150 mg/kg (Goldner, 1945), rabbit 150 mg/kg (Lisewski and Mohnike, 1959 a), pigeon 125–200 mg/kg (Goldner, 1945), and monkey 150–300 mg/kg (Goldner, 1945; Ramfjord, 1952). These doses are valid only under the experimental conditions used. Subcutaneous diabetogenic doses are usually 150–250 mg/kg for rodents and intraperitoneal doses are 200–300 mg/kg. The lethal dose bears no constant relationship to the diabetogenic dose, but is generally around twice that selectively eliminating \(\beta\)-cells.

Relation of Structure to Diabetogenic Activity

There are very few derivatives of alloxan or related compounds which are diabetogenic. Methyl-, ethyl-, and propylalloxan are diabetogenic, but it becomes progressively harder to induce diabetes because the toxicity increases with the length of the side chain (Brückmann and Wertheimer, 1947). Thus butyl-, phenyl-, and benzylalloxan cannot be given in doses sufficient to produce diabetes, but possibly they possess the ability to destroy \(\beta\)-cells. Substitution at both N atoms, however, results in a loss of
activity, and it has been postulated that this is perhaps due to the lack of enolization (Hidy, 1946), but it could also be due to greater instability and toxicity (Bernhard et al., 1947). Dialurate is diabetogenic since it can be oxidized to alloxan in the body (Siliprandi and Pisati, 1949), but if this is prevented by the administration of cysteine, it becomes inactive (Lazarow et al., 1948). Alloxantin readily breaks down to alloxan and hence is diabetogenic (Brada, 1949). Urate has been reported to produce diabetes in massive doses under appropriate conditions, and metabolism to alloxan has been thought to be responsible, but the present evidence is against this. Some metal chelates of alloxan produce an initial hyperglycemia, but whether β-cell destruction can occur is not known (Lange and Foye, 1956).

The following substances have been found to be nondiabetogenic: isatin, ninhydrin, and benzoyleneurea (Hidy, 1946); alloxanate, barbiturate, murexide, mesoxalate, oxalurate, and parabanate (Jacobs, 1937); alloxantin, murexide, isatin, and ninhydrin (Brückmann and Wertheimer, 1947); caffeine and phenoldialurate (Bernhard et al., 1947); violuric acid and uramil (Brückmann and Wertheimer, 1945); and uridine (Lazarow, 1954 a).

Brückmann and Wertheimer (1947) pointed out that the reactive portion of the alloxan molecule seems to be the central hydrated carbonyl of the $-\text{CO} - \text{C(OH)}_2 - \text{CO}$— moiety, and that alteration of this abolishes the diabetogenic activity. Dehydroascorbate possesses this grouping and is somewhat diabetogenic. However, as discussed previously, it is not certain that the 5-CO group is hydrated. Also, any change at the 5-position would not only change its properties, but alter the rest of the molecule; e.g., the quinonoid structure would become impossible. The inactivity of $N$, $N'$-dimethylalloxan, which possesses the above grouping, is difficult to explain,
and it may be noted that ninhydrin is non-diabetogenic. Lazarow (1954 a) has given a full discussion of the structural requirements for diabetogenic activity, but at present it is impossible to formulate a specific grouping which will produce diabetes. Certain data indicate the importance of the —NH—CO—NH—CO— moiety and its enolized tautomer —N=C(OH)— —N=C(OH)— but a number of compounds have this grouping and are inactive. Little attention seems to have been paid to the possibility that the quinonoid structure of alloxan is the reactive form. It may be noted that none of the inactive compounds can assume such a structure.

**Effects of Alloxan on Tissue Thiols**

A relationship between the diabetogenic action of alloxan and its reaction with glutathione or other thiols has been postulated, particularly by Lazarow (1954 a). There is no doubt that alloxan injected intravenously brings about a profound decrease in blood glutathione. Leech and Bailey (1945) showed that the injection of 200 mg/kg of alloxan into rabbits reduces the blood glutathione 50–100%, the maximal effect occurring at 1–2 min, and from the parallel disappearance of alloxan and glutathione they concluded that reaction between them took place. This was confirmed by Brückmann and Wertheimer (1947) in the rat. However, reduction of blood glutathione cannot be the mechanism of the effect on the β-cells. For one thing, the glutathione is mainly in the erythrocytes and its loss there would not so rapidly affect other tissues, and also several substances lower blood glutathione and do not cause diabetes, examples being ninhydrin (Brückmann and Wertheimer, 1947), iodoacetate, and iodoacetamide (Hultquist, 1958). The effects on blood glutathione merely demonstrate the ability of alloxan to react rapidly with glutathione. One might expect that this is a simple oxidation of glutathione, but Bhattacharya et al. (1956) showed that alloxan lowers only GSH, the GSSG level remaining unaffected, so that there is a loss of total glutathione. This points to a combination of alloxan with glutathione, possibly to form Complex 305 or a related substance.

Of more importance are the effects of alloxan on tissue thiols and particularly the changes possibly occurring in the β-cells. DeCaro and Rovida (1937) reported that alloxan produces moderate falls in liver and intestinal glutathione within 10 min. There is a decrease in liver and kidney glutathione during severe diabetes resulting from alloxan, but these results were obtained at 48 hr and, as Houssay et al. (1947) pointed out, tissue thiols fall after pancreatectomy. Thus only the early changes are of significance for our purpose. Brada (1951) determined the glutathione level in various tissues of the rat following injection of alloxan (see accompanying tabulation) and believed the drops in adrenals, pancreas, and liver to be significant, although it seems likely that the renal effect is also real. Except for the adre-
nals, the levels are not only restored but appear to be higher at 90 min. More striking effects might have been noted if a shorter interval than

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% Change of GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45 min</td>
</tr>
<tr>
<td>Adrenals</td>
<td>- 96</td>
</tr>
<tr>
<td>Pancreas</td>
<td>- 47</td>
</tr>
<tr>
<td>Kidney</td>
<td>- 35</td>
</tr>
<tr>
<td>Liver</td>
<td>- 24</td>
</tr>
<tr>
<td>Brain</td>
<td>- 2</td>
</tr>
<tr>
<td>Spleen</td>
<td>+ 3</td>
</tr>
<tr>
<td>Heart</td>
<td>+ 4</td>
</tr>
<tr>
<td>Muscle</td>
<td>+ 12</td>
</tr>
</tbody>
</table>

45 min had been used. This was done by Binet et al. (1958) and the initial decreases in glutathione are indeed greater (see accompanying tabulation).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% Change of GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-5 min</td>
</tr>
<tr>
<td>Muscle</td>
<td>- 40</td>
</tr>
<tr>
<td>Blood</td>
<td>- 34</td>
</tr>
<tr>
<td>Heart</td>
<td>- 24</td>
</tr>
<tr>
<td>Lungs</td>
<td>- 16</td>
</tr>
<tr>
<td>Kidneys</td>
<td>- 11</td>
</tr>
<tr>
<td>Liver</td>
<td>+ 9</td>
</tr>
</tbody>
</table>

These results are not only quite different from those of Brada, but with respect to blood and liver changes are different from those of all other workers.

The results obtained on the alloxan-induced changes in pancreatic thiols are unfortunately divergent. We have seen that Brada (1951) observed a significant fall in pancreatic glutathione 45 min following the administration of alloxan, but Hultquist (1956) could find no changes. It must be realized that the β-cells constitute only about 0.5–1% of the total mass of the pancreas and, hence, analyses of the entire organ may be meaningless with respect to the action on the β-cells. MacDonald (1959) used Bennett’s red SH reagent to determine the SH group content of acinar and islet tissue, and showed that alloxan lowers the β-cell SH content relative to acinar tissue, the difference being evident at 15 min and marked after 2 hr. Falk-
mer (1961) also reported a fall in islet glutathione in the sculpin, the islets here being susceptible to isolation and analysis. It would thus seem definite that alloxan can react with the SH groups of β-cells.

Substances Antagonizing the Diabetogenic Action of Alloxan

There are many substances which delay, decrease, or abolish the action of alloxan on the β-cells when they are administered previously, or occasionally with the alloxan. Our interest in such protective substances is in the information they may shed on the mechanisms by which alloxan acts. Some protectors are listed in Table 4-1; others not listed include dimer-caprol, propanol, isopropanol, o-phenylenediamine, bisulfitte, thioglycolate, yeast pentose nucleotides, methionine, norepinephrine, phenylephrine, 3,4-diaminotoluene, and 1,2-dimethyl-4-amino-5-(ribitylamino)benzene. In many cases it is a matter of the simple inactivation of the alloxan before it gets to the islets (e.g., glutathione, cysteine, borate, ascorbate, 1,2-dienolglucose, and metal ions). Nitrite and p-aminopropiophenone both elevate blood glutathione and favor the formation of methemoglobin, so that the effect is secondary but similar. In not one of these cases do we learn much of interest, except that thiols must be administered not longer than 5 min before the alloxan if protection is to be observed (Lazarow, 1946), and this shows that the thiols are not reactivating alloxan-inhibited systems and that the important effects of alloxan on the β-cells occur within 5 min, a conclusion substantiated from other evidence. Epinephrine and other pressor amines apparently act by producing pancreatic vasoconstriction so that the injected alloxan cannot reach the islets, since phenotolamine (Regitine) prevents both vasoconstrictive and antidiabetogenic activities (Meade and Klitgaard, 1960). Deoxycorticosterone presumably acts through alteration of blood electrolytes, since Na⁺ deficiency has been shown to increase the sensitivity to alloxan due to a lowered blood level of glutathione (Grunert and Phillips, 1949). The protections by nicotinamide, barbiturate, and glucose are more interesting.

It had been shown that alloxan can compete with NAD for certain yeast apoenzymes (see page 400), so Lazarow et al. (1950) attempted to combat the diabetogenic action of alloxan by injecting NAD, but without success, due probably to the failure to penetrate into the cells. However, nicotinamide injected up to 1 hr before the alloxan protects quite well at a dose of 915 mg/kg (7.5 millimoles/kg). Lazarow and his group presented four possible mechanisms for the protection: (1) nicotinamide is methylated to N-methylnicotinamide, which reacts with aldehydes and might react with alloxan, (2) the conversion of nicotinamide to N-methylnicotinamide removes the methyl group from methionine, leaving homocysteine, which might inactivate alloxan, (3) nicotinamide is also converted in part to NAD(H) and this might protect the enzymes from alloxan, or even the
Table 4-1
SUBSTANCES WHICH PROTECT ANIMALS FROM THE DIABETOGENIC ACTION OF ALLOXAN

<table>
<thead>
<tr>
<th>Protective substance</th>
<th>Dose (mg/kg)</th>
<th>Animal</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Aminopropiophenone</td>
<td>0.43</td>
<td>Rat</td>
<td>Vollmer et al. (1954)</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>0.25</td>
<td>Dog</td>
<td>Merlini (1951)</td>
</tr>
<tr>
<td>Barbituric acid</td>
<td>150</td>
<td>Rat</td>
<td>Martinez (1955)</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>Dog</td>
<td></td>
</tr>
<tr>
<td>Benzamide</td>
<td>125</td>
<td>Rat</td>
<td>Janes and Schueler (1955)</td>
</tr>
<tr>
<td>Borate</td>
<td>—</td>
<td>Rat</td>
<td>Kuhn and Quadbeck (1948)</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>20</td>
<td>Dog</td>
<td>Simoes and Osswald (1955)</td>
</tr>
<tr>
<td>Co⁺⁺</td>
<td>1</td>
<td>Rat</td>
<td>Lazarow and Patterson (1951)</td>
</tr>
<tr>
<td>Cysteine</td>
<td>304</td>
<td>Rat</td>
<td>Lazarow (1946)</td>
</tr>
<tr>
<td>Deoxycorticosterone</td>
<td>—</td>
<td>Rat</td>
<td>Grunert and Phillips (1949)</td>
</tr>
<tr>
<td>1,2-Dienoglucose</td>
<td>1:1ᵃ</td>
<td>Rat</td>
<td>Nath and Bhattathiry (1956)</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>—</td>
<td>Rat</td>
<td>Kass and Waisbren (1945)</td>
</tr>
<tr>
<td>Ethionine</td>
<td>—</td>
<td>Rat</td>
<td>Gambassi and Del Gatto (1956)</td>
</tr>
<tr>
<td>Fe⁺⁺</td>
<td>6</td>
<td>Rat</td>
<td>Lazarow and Patterson (1951)</td>
</tr>
<tr>
<td>Fructose</td>
<td>—</td>
<td>Rat</td>
<td>Bhattacharya (1953)</td>
</tr>
<tr>
<td>D-Glucose</td>
<td>2000</td>
<td>Dog</td>
<td>Arteta et al. (1954)</td>
</tr>
<tr>
<td></td>
<td>1080</td>
<td>Rat</td>
<td>Carter and Younathan (1962)</td>
</tr>
<tr>
<td>Glutathione</td>
<td>700</td>
<td>Rat</td>
<td>Lazarow (1946)</td>
</tr>
<tr>
<td>Mannose</td>
<td>—</td>
<td>Rat</td>
<td>Bhattacharya (1953)</td>
</tr>
<tr>
<td>3-Methyl-D-glucose</td>
<td>1000</td>
<td>Rat</td>
<td>Carter and Younathan (1962)</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>125</td>
<td>Rat</td>
<td>Janes and Schueler (1955)</td>
</tr>
<tr>
<td></td>
<td>915</td>
<td>Rat</td>
<td>Lazarow et al. (1950)</td>
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<td>Nitrite (Na)</td>
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<td>Rat</td>
<td>Vollmer et al. (1954)</td>
</tr>
<tr>
<td>d-Penicillamine</td>
<td>1000</td>
<td>Mouse</td>
<td>Matsuda and Makino (1961)</td>
</tr>
<tr>
<td>Prophylene glycol</td>
<td>4000</td>
<td>Rat</td>
<td>Janes and Schueler (1955)</td>
</tr>
<tr>
<td>Thiouracil</td>
<td>—</td>
<td>Dog</td>
<td>Houssay (1950)</td>
</tr>
<tr>
<td>Zn⁺⁺</td>
<td>6.5</td>
<td>Rat</td>
<td>Lazarow and Patterson (1951)</td>
</tr>
</tbody>
</table>

ᵃ Dose equimolar with alloxan.
nicotinamide might protect directly by binding at the NAD sites, or (4) the excess NADH formed from nicotinamide might reduce the alloxan to dialurance. Younathan (1962) showed that the microsomal fraction of kidney and liver homogenates is necessary for the inhibitory action of alloxan on cycle oxidations, and postulated that alloxan might be converted to some amobarbital-like substance capable of inhibiting NAD-dependent oxidations. Thus nicotinamide could protect either by increasing the NAD levels in the \( \beta \)-cells or by providing excess NAD to replace that lost from hyperpermeable mitochondria. The interest in the protective action of barbiturate is that the structure is similar to alloxan. Barbiturate is not diabetogenic but it may bind to the sites with which alloxan reacts and so protect the \( \beta \)-cells (Martinez, 1955). If this is so, it indicates that alloxan does not react rather nonspecifically with membrane or enzyme SH groups, but attacks some site with a complementary structure.

The protection exerted by glucose is especially interesting in that it might provide evidence for the mechanism by which alloxan modifies \( \beta \)-cells. Kass and Waishbren (1945) noted that feeding or injecting glucose 6 hr before the administration of alloxan reduces the susceptibility of previously starved rats to alloxan; however, no effect was seen when the glucose was injected 1 hr before the alloxan, which makes it likely that the protection observed was of a different kind than studied subsequently. It remained for Bhattacharya (1953, 1954) to demonstrate that glucose protects when injected immediately before alloxan, but not when given afterward. There is no evidence for reaction between glucose and alloxan. Mannose is about one half as effective as glucose, and fructose is about one seventh as effective. A competitive-like relationship was established and it was thought that alloxan may inhibit hexokinase, and hence glucose uptake and utilization, this enzyme being protected by the administered glucose. Arteta et al. (1954) confirmed this protective action in dogs and suggested that the concentration of glucose around the \( \beta \)-cells may control the sensitivity to alloxan.

The following theories for the protection have been advanced: (1) the direct protection of hexokinase mentioned above, (2) the metabolism of glucose provides something, perhaps high-energy substances, which are protective (Villar-Palasi et al., 1957), and (3) the enhancement of the release of epinephrine by glucose (Vollmer et al., 1960). Despite the fact that Villar-Palasi et al. (1957) found hexokinase from dog pancreas to be fairly readily and rapidly inhibited by alloxan, and glucose to exert some protection against alloxan, they concluded that this is not the mechanism, mainly because the ratio of the protective abilities of glucose, mannose, and fructose does not correspond to the affinities of these sugars for hexokinase (it might be noted that in such discussion there is often some confusion between affinities and phosphorylation rates of the various sugars).
Even though the hexokinase studied was from pancreas, it is likely that it is not primarily \( \beta \)-cell hexokinase, which might be more sensitive to alloxan. Nevertheless, this hexokinase appears to be quite significantly inhibited in the absence of glucose by 0.1–0.5 mM alloxan.* Carter and Younathan (1962) found that 3-methyl-D-glucose also protects, but \( \alpha \)-D-methylglucoside, malate, lactate, and a fumarate-pyruvate mixture do not. Release of epinephrine seems doubtful on various grounds, but they showed that phentolamine, which blocks the vasoconstrictive action of epinephrine, does not alter the protective ability of glucose. The results with 3-methyl-D-glucose are difficult to explain since it is not metabolized (at least not by most tissues, although possibly by \( \beta \)-cells), and hence the theory that glucose provides energy in some form to protect is invalidated. Further discussion of these points will be found in the section summarizing the possible mechanisms by which alloxan acts.

Effect of Alloxan on Islet Cell Tumors

Once the selective destructive action of alloxan on \( \beta \)-cells was discovered, it was obvious that alloxan might be clinically useful in islet cell tumors. Brunschwig et al. (1944) and Brunschwig and Allen (1944) reported the treatment of an insulin-producing carcinoma and could not produce necrosis of the malignant cells, although the attacks of hyperinsulinism were abolished for some time. Conn et al. (1947) intravenously injected 50 mg/kg alloxan each day for 9 days and then 100 mg/kg/day for a while, 15 days after which the insuloma was removed. There had been no demonstrable effect of alloxan on the \( \beta \)-cells and insulin continued to be secreted during the time of treatment and presurgery. They noted that normal islet tissue was damaged by the alloxan. Böttger et al. (1952) also treated a patient with an islet cell adenoma with increasing intravenous doses of 8–71 mg/kg over 2 months, but no effect was noticed and the patient died in hyperinsulin coma. Normal \( \beta \)-cells were again found to be damaged. Unfortunately, it appears that tumorous \( \beta \)-cells are quite resistant to alloxan, which probably indicates that the systems primarily responsible for insulin synthesis and release are not involved in the immediate action of alloxan, but the resistance could be explained if the \( \beta \)-cell plasma membranes are altered by malignancy.

Natural Occurrence of Alloxan

The hypoglycemia following administration of alloxan was attributed by Dunn et al. (1943 a) to stimulation of the islets to release insulin, and they

* These experiments were unfortunately not reported, but 4 mM alloxan inhibits this hexokinase 93% in the absence of glucose and 58% in the presence of 5 mM glucose. Since 0.5 mM alloxan inhibits 25% in the presence of glucose, it is likely that a good deal more depression would be noted without glucose.
wondered if alloxan might be a circulating hormone controlling insulin secretion. When the diabetogenic action was soon established, it was suggested by several workers that hyperalloxanemia might be a contributory factor in the genesis of diabetes. The presence of alloxan in tissues would not be surprising inasmuch as it could arise from the metabolism of the pyrimidines and purines, and, indeed, is chemically obtained from urate. Alloxan is formed from urate in leucocytes in the presence of hydrogen peroxide due to the action of myeloperoxidase (Soberón and Cohen, 1963). Much work has been done in attempting to determine the levels of alloxan in the blood under various conditions, but the results are discordant, possibly due to the unsatisfactory analytical methods often used. One would certainly never expect to find high concentrations in any tissue because of the instability of alloxan; we shall see that injected alloxan is mainly destroyed in a few minutes. Abderhalden (1916) claimed that the frequent concurrence of gout and diabetes might be interpreted to mean that abnormal purine metabolism could give rise to sufficient alloxan to be diabetogenic. Numerous studies in the rat, rabbit, dog, horse, and man have shown that the normal alloxan content of blood is probably between 0.015 and 0.2 mg% (Archibald, 1945; Schioler, 1948; Huisman et al., 1950; Loubatières and Bouyard, 1951 a; Sobotka and Luisada-Opper, 1954), this corresponding to 0.001-0.014 m\(MU\) alloxan. Some, for example Karrer et al. (1945), could detect no alloxan in the blood or urine of diabetics. There may be some question as to the validity of the analyses since the specificities of the methods are often doubtful, and possibly the normal values given above do not refer to alloxan. In any event, the alloxan concentration in normal blood is very low.

With respect to the etiology of diabetes, the real problem is whether alloxan occurs at elevated levels in the blood or islets under abnormal conditions. Lang (1866) reported a patient who voided a yellow urine which turned reddish blue on standing, and interpreted this as due to alloxan or a precursor (possibly dialurate) forming murexide. As far as I know, no further cases of this type have been recorded and it may be that alloxan was not involved. Tipson and Ruben (1945) stated that abnormal overproduction of alloxan or failure to destroy it might lead to abnormally high levels and claimed to have detected alloxan in the livers of various species, using the barium test in deproteinized extracts (Ruben and Tipson, 1945). These results are not convincing. The question of the conversion of urate to alloxan in animals has never been satisfactorily answered, but Grunert and Phillips (1951) could find no alloxan formed from urate in liver homogenates, and Lee and Stetten (1952) detected no labeled alloxan following administration of urate-1,3-\(^{14}\)C. Soberón and Cohen (1957) claimed, however, that incubation of rat neutrophils with urate-2-\(^{14}\)C leads to around 10% conversion to alloxan. Seligson et al. (1951) reasoned
that alloxan is rapidly broken down to alloxanate in blood and hence one
should perhaps analyze for the latter to detect if alloxan is produced in
the tissues. Hydrolysis of alloxanate gives oxomalonate and this can be
detected as a dinitrophenylhydrazone. Alloxanate was found in the urine
of normals and diabetics, but the levels were not stated.

Schieler (1948) administered glucose orally to fasted rats and found the
blood alloxan to be increased some 50-fold to around 0.07 mM. This could
not be confirmed in rats by Huisman et al. (1950) or in the dog and man
by Sobotka and Luisada-Opper (1954). However, Loubatières and Bou-
yard (1951 a, b) found a similar 50-fold increase when the glucose is given
orally, but essentially no effect when the glucose is injected parenterally.
They believe that alloxan arises in the intestine and depends on glucose
absorption, since isolated intestine releases alloxan when treated with glu-
cose. Hyperalloxanemia was claimed to occur in experimental diabetes,
due to the elevated glucose levels, and it was suggested that diabetes might
occasionally be produced by release of alloxan during periods of hyper-
glycemia (Loubatières, 1954).

The interesting problem of the possible role of alloxan in the genesis
of diabetes has thus not been settled. It is conceivable that a disturbance
in purine metabolism in the $\beta$-cells could lead to a localized high concen-
tration of alloxan, but this would be very difficult to detect.

**FATE OF ALLOXAN IN ANIMALS**

Alloxan is unstable in aqueous solutions and even more so in the pres-
ence of tissues or when injected into animals. Any direct effects of al-
loxan must therefore be exerted rapidly. Early work showed that alloxan
is metabolized completely in the body, alloxanate, alloxantin, parabanate,
and the red murexide appearing in the urine (Koehme, 1894; Lusini, 1894;
Cerecedo, 1931), and that application of high concentrations of alloxan to
various tissues leads to a red color, due presumably to murexide (Labes
and Freisburger, 1930; Lieben and Edel, 1932, 1933). We have seen that
alloxan is rapidly destroyed in blood and so it is not surprising that in-
travenously injected alloxan disappears from the blood within 3-5 min
(Leech and Bailey, 1945; Vekslers, 1956). It has thus been concluded that
the effect of alloxan on the $\beta$-cells must occur within 5 min after injection.
Some of the alloxan is converted to alloxanate (Paley et al., 1953) but
an appreciable fraction must be reduced to dialurate, which could be
reoxidized to alloxan in certain tissues, possibly by the cytochrome system.
The level of alloxan in a tissue might then be determined in part by the
balance between oxidizing and reducing potencies. The results of Siliprandi
(1948) are somewhat different from those obtained by other workers
(Fig. 4-1), in that a much slower loss in the blood was observed. It is also
surprising that the tissue levels remain so high after 30 min. He used the o-phenylenediamine reaction to determine alloxan and it may be that some of the metabolic products contribute. It is often stated that alloxan is destroyed mainly in the liver but I know of no evidence for this; indeed, the results of Fište et al. (1957) indicate that various tissues are able to metabolize alloxan.

The use of radioactively labeled alloxan has provided further information on the distribution and fate of alloxan. Alloxan-1,3-N\textsuperscript{15} injected in diabetogenic doses in rats and rabbits was recovered mainly in the nonprotein fractions of tissues, after 1 hr highest concentration occurring in the kidney (Lee and Stetten, 1952). The pancreatic level is always rather low. Only two thirds of the injected isotope is excreted in the urine within 3 days, so significant amounts of the alloxan metabolites must be incorporated into the tissues. Injection of alloxan-5-C\textsuperscript{14} or alloxan-2-C\textsuperscript{14} gives a much higher recovery (90–95\%) in the urine during the first day (Janes and Winnick, 1952). Almost no C\textsuperscript{14}O\textsubscript{2} appears, indicating that alloxan is not catabolized. About 80\% of the urinary activity is probably alloxanate.
The similar distribution of the 2-$^{14}$C and 5-$^{14}$C alloxans shows that the ureide and malonic acid portions of the molecule are not split apart. Again, only low activity was found in the pancreas. No evidence has emerged from any of this work that alloxan is accumulated in the pancreas, but gross analyses may not reveal the levels in the $\beta$-cells. However, Landau and Renold (1954) obtained autoradiographs of tissues from rats given alloxan-2-$^{14}$C and could find only a rather uniform distribution in the tissues, the islets containing no more than lung, liver, and spleen. Since the animals were sacrificed 5 min after the alloxan injection, at a time when alloxan is presumably exerting its action on the $\beta$-cells, it would appear that this is valid evidence against selective uptake by the islets.

**EFFECTS ON ENZYMES**

Many of the results on enzyme inhibition are difficult to interpret because insufficient attention has been paid to (1) the rapid disappearance of alloxan during the incubation with the enzyme, (2) the fall in the pH accompanying the formation of alloxanate, and (3) the possibility of inhibition by the products of alloxan breakdown. Under the usual conditions of enzyme study, and at concentrations of alloxan below 5 mM, it is likely that the action of alloxan on the enzyme is confined to a period not longer than 2–3 min. Increases of inhibition after this could be due to secondary changes in the enzyme or to effects exerted by the alloxan products. Alloxan may react with enzymes in the ways outlined for proteins (page 379) and, in addition, may inhibit by inactivating cofactors or by competing with substrates or coenzymes because of structural similarity. Interest in the inhibition of enzymes by alloxan lies either in the characterization of functional SH groups or in the attempt to find a susceptible enzyme whose inactivation would explain the diabetogenic activity. Some inhibitions are summarized in Table 4-2. Probably some of these inhibitions would be much greater if care had been taken to expose the enzymes to freshly dissolved or neutralized alloxan.

Alloxan can presumably either oxidize enzyme SH groups or react with them to form a complex. If the inhibition is entirely due to oxidation, it should be reversible upon adding cysteine or glutathione, provided that no secondary inactivation has occurred subsequent to the formation of disulfide bonds. In the earliest work on enzymes, Purr (1935) claimed that the SH groups of papain and cathepsin are oxidized without affecting the protein structure, since activity could be restored upon reduction. In most instances, reactivation is partial — as for succinate dehydrogenase (Hopkins et al., 1938), fructose-1,6-diphosphatase (Walsh and Walsh, 1948), and the liver acetylating enzyme (Cooperstein and Lazarow, 1958) — so it is difficult to interpret the results. That part of the inhibition due to
### Table 4.2

**Inhibition of Enzymes by Alloxan**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Alloxan (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aconitase</td>
<td>Pigeon muscle</td>
<td>10</td>
<td>78</td>
<td>Krebs and Eggleston (1944)</td>
</tr>
<tr>
<td>Adenosinetriphosphatase</td>
<td>Guinea pig brain</td>
<td>1</td>
<td>17</td>
<td>Gore (1951)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat brain</td>
<td>0.5</td>
<td>44</td>
<td>Gordon (1953)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>d-Amino acid oxidase</td>
<td>Lamb kidney</td>
<td>3</td>
<td>0</td>
<td>Frisell <em>et al.</em> (1956)</td>
</tr>
<tr>
<td>alpha-Amylase</td>
<td>Wheat</td>
<td>10</td>
<td>0</td>
<td>Ghosh (1958)</td>
</tr>
<tr>
<td>Arginase</td>
<td>Rat liver</td>
<td>21</td>
<td>Stim 25</td>
<td>Purra and Weil (1934)</td>
</tr>
<tr>
<td>Arylamine transacetylase</td>
<td>Pigeon liver</td>
<td>0.25</td>
<td>54</td>
<td>Wrenshall (1957)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.5</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pigeon liver</td>
<td>0.05</td>
<td>60</td>
<td>Cooperstein and Lazarow (1958)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>80</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0.25</td>
<td>93</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>Carbonic anhydrase</td>
<td>Spinach leaves</td>
<td>5</td>
<td>7</td>
<td>Chiba <em>et al.</em> (1954 c)</td>
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<tr>
<td>Choline acetylase</td>
<td>Frog brain</td>
<td>0.007</td>
<td>12</td>
<td>Torda and Wolff (1946 b)</td>
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<tr>
<td></td>
<td></td>
<td>0.07</td>
<td>20</td>
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</tr>
<tr>
<td>Enzyme</td>
<td>Source</td>
<td>V</td>
<td>50</td>
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</tr>
<tr>
<td>-----------------------------------------</td>
<td>-------------------------</td>
<td>----</td>
<td>----</td>
<td></td>
</tr>
<tr>
<td>Choline dehydrogenase</td>
<td>Rat liver</td>
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<td>Choline oxidase</td>
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<tr>
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<td>Erythrocytes</td>
<td>23.4</td>
<td>Inh</td>
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<tr>
<td></td>
<td></td>
<td>14</td>
<td>&gt;50</td>
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<td>β-Fructofuranosidase</td>
<td>Yeast</td>
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<td>0</td>
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<td>Fructose-1,6-diphosphatase</td>
<td>Liver</td>
<td>2.5</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Galactonolactone dehydrogenase</td>
<td>Peas</td>
<td>1</td>
<td>25</td>
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</tr>
<tr>
<td>Glucose-6-phosphatase</td>
<td>Rabbit liver</td>
<td>14</td>
<td>30</td>
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<tr>
<td>Hexokinase</td>
<td><em>Bacillus sp.</em></td>
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<td>30</td>
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<td></td>
<td></td>
<td>0.32</td>
<td>50</td>
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<tr>
<td></td>
<td></td>
<td>1</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td>Wheat germ</td>
<td>Soluble</td>
<td>5</td>
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<tr>
<td></td>
<td>Particulate</td>
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<td>19</td>
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<td>Potato</td>
<td>2.86</td>
<td>0</td>
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<tr>
<td></td>
<td>Rabbit muscle</td>
<td>1.25</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat liver</td>
<td>2.87</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dog pancreas</td>
<td>0.5</td>
<td>25</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>30</td>
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</tr>
<tr>
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<td>2</td>
<td>40</td>
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<td></td>
<td></td>
<td>4</td>
<td>58</td>
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</tr>
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Gordon and Quastel (1948)
Rothschild *et al.* (1954)
Nachmansohn and Lederer (1939)
Becker and Rauschke (1951)
Robuschi (1952)
Gemmill and Bowman (1950)
Walsh and Walsh (1948)
Mapson *et al.* (1954)
Broh-Kahn *et al.* (1948)
Sato *et al.* (1956)
Saltman (1953)
Griffiths (1949)
Griffiths (1949)
Bhattacharya (1959)
Villar-Palasi *et al.* (1957)
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<th>% Inhibition</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>Pig heart</td>
<td>4</td>
<td>0</td>
<td>Lotspeich and Peters (1951)</td>
</tr>
<tr>
<td>Lipase</td>
<td>Pancreas</td>
<td>1</td>
<td>0</td>
<td>Wills (1960)</td>
</tr>
<tr>
<td>NAD nucleosidase</td>
<td>Rabbit erythrocytes</td>
<td>—</td>
<td>0</td>
<td>Alivisatos et al. (1956)</td>
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<tr>
<td>Nitrate reductase</td>
<td><em>Achromobacter fischeri</em></td>
<td>5</td>
<td>0</td>
<td>Sadana and McElroy (1957)</td>
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<tr>
<td>Phosphatase (alkaline)</td>
<td>Pig kidney</td>
<td>2</td>
<td>50</td>
<td>Burgen and Lorch (1947)</td>
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<tr>
<td></td>
<td></td>
<td>10</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rat intestine</td>
<td>0.1</td>
<td>2</td>
<td>Larralde and Ponz (1949)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>Phosphoglucomutase</td>
<td>Rabbit muscle</td>
<td>2</td>
<td>35</td>
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<td></td>
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<td></td>
<td>Rabbit liver</td>
<td>14</td>
<td>30</td>
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<td>3-Phosphoglyceraldehyde dehydrogenase</td>
<td>Rabbit muscle</td>
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<td>Phosphoprotein phosphatase</td>
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<td>Sundararajan and Sarma (1954)</td>
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<td>Phosphorylase</td>
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<td></td>
<td>Liver</td>
<td>30</td>
<td>0</td>
<td>Ševela and Talafant (1951)</td>
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<td>Pyrophosphatase</td>
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<td>0.1</td>
<td>24</td>
<td>Gordon (1950)</td>
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### EFFECTS ON ENZYMES

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<th>Source</th>
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<th>pH 6.6</th>
<th>pH 7.0</th>
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<th>pH 8.0</th>
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<td>Rat brain</td>
<td>0.5</td>
<td>5</td>
<td>23</td>
<td>10</td>
<td>21</td>
<td>100</td>
<td></td>
<td>Gordon (1953)</td>
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<td>Guinea pig brain</td>
<td>1</td>
<td></td>
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<td></td>
<td></td>
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<td>Gore (1951)</td>
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<td>Human erythrocytes</td>
<td>2</td>
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<td></td>
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<td>Naganna and Menon (1948)</td>
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<td>Yeast</td>
<td>0.5</td>
<td>15</td>
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<td></td>
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<td></td>
<td>Kensler et al. (1942 a)</td>
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<td>Yeast</td>
<td>1.5</td>
<td>10</td>
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<td>Kuhn and Beinert (1947)</td>
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<td>12.5</td>
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<td>Stoppani et al. (1952)</td>
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<td>Human erythrocytes</td>
<td>5</td>
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<td>Solvonuk and Collier (1955)</td>
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<td>Rhodanese</td>
<td>Beef liver</td>
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<td>Sedoheptulose kinase</td>
<td><em>Bacillus</em> sp.</td>
<td>0.1</td>
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<td>Ebata et al. (1955)</td>
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<td>Rabbit muscle</td>
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<td>Hopkins et al. (1938)</td>
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<tr>
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<td>Rat liver</td>
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<td></td>
<td></td>
<td>Bhattacharya (1954)</td>
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<td>Rat kidney</td>
<td>3</td>
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<td>Jack bean</td>
<td>1</td>
<td>62</td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td>10</td>
<td>72</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Xanthine oxidase</td>
<td>Milk</td>
<td>5</td>
<td>31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bruns (1954)</td>
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</tbody>
</table>
inactivation of coenzyme A could be reversed, but the inhibition directly on the acetylating enzyme could not. The inactivation of rat liver hexokinase could not be reversed at all with cysteine and it was concluded that something more than simple oxidation must be involved (Bhattacharya, 1959). Siliprandi and Daghetta (1950) noted that alloxan inhibits urease more strongly in oxygen than in nitrogen, and thought that this pointed to an oxidation mechanism.

The inhibition is usually noncompetitive — as with renal phosphatase (Burgen and Lorch, 1947), xanthine oxidase (Bruns, 1954), and urease (Gray et al., 1959) — but in some cases one finds a kinetic competition, in that protection is exerted by substrates or coenzymes — for example, fructose-6-P and ATP protect phosphofructokinase (Engelhardt and Sakov, 1943), glucose protects pancreatic hexokinase (Villar-Palasi et al., 1957), and NAD protects a yeast apozymase system (Kensler et al., 1942 b). The inhibition of urease by alloxan is perhaps partially competitive, due to the urea moiety, but this is complicated by the fact that alloxanate is a more potent inhibitor than alloxan and may be responsible for the inhibition when alloxan is used.

Another type of effect of alloxan on dehydrogenases must be considered. Dixon and Zerfas (1940) showed that alloxan could act as a hydrogen acceptor in several dehydrogenase systems when used at a low concentration. Unlike methylene blue or quinone, it does not require NAD to be reduced to dialurate. Keleti (1958) determined the equilibrium constant

\[ K = \text{(dialurate) (acetaldehyde)/(alloxan) (alcohol)} = 1.24 \times 10^{-11} \]

using the alcohol dehydrogenase system, and felt that alloxan might combine with the Zn\(^{++}\) ions on the enzyme in the same manner as NAD. Whatever the mechanism, it is clear that alloxan can function in certain oxidation-reduction sequences and thereby possibly stimulate oxygen uptake.

Succinate dehydrogenase is inhibited more potently by alloxan the lower the pH (Table 4-2) (Klebanoff, 1955). It is not known whether this is a real pH effect on the reaction of alloxan with the enzyme, or merely an expression of the fact that alloxan is more stable at lower pH. The inhibition is found to be progressive over 30 min when 20 mM alloxan is used, but this is due to the fall in pH during the first 1–2 min; if this fall is prevented, the inhibition does not increase following the rapid initial depression, showing that it is an acid effect. It must be emphasized again that pH control is especially important in work with alloxan.

We come now to the important question: Have any enzymes been found sufficiently sensitive to alloxan to be seriously considered as involved in the diabetogenic action? Lazarow (1954 b) has estimated that in the rat a diabetogenic dose of 40 mg/kg intravenously would give a maximal alloxan concentration of 0.5 mM. Other species require higher doses, so that
2-3 times this concentration might be achieved in the cat or rabbit, for example. It is very difficult to estimate the peak alloxan concentration at the \( \beta \)-cells following injections, since it depends to a great extent on the rate of injection, and also on the site of the injection and the rate of destruction. It may well be that peak concentrations higher than that given by Lazarow occur, although much lower levels are equally possible, particularly after subcutaneous or intraperitoneal injections. Inspection of Table 4-2 shows that few enzymes are markedly inhibited in this range. Some, like choline acetylase, pyrophosphatase, and urease, which are quite potently inhibited, could scarcely be implicated in the diabetogenic action. Certain ATPases and kinases are sensitive and might be involved. 3-Phosphoglyceraldehyde dehydrogenase appears to be quite sensitive but the data are insufficient to evaluate this. The high susceptibility of the liver acetylating enzyme indicates a possible action on transacetylations involving coenzyme A, but it is difficult to understand how such an inhibition could result in such rapid cytological changes in the \( \beta \)-cells. Certain cycle enzymes are the most sensitive to alloxan of the enzymes studied (Younathan, 1962). Some inhibition of the oxidation of pyruvate-fumarate mixtures in rat kidney mitochondria is evident at 0.003 mM alloxan, and 0.03 mM seems to inhibit completely. The same is true for the oxidation of pyruvate and malate. The same enzymes in liver mitochondria are much less sensitive to alloxan, so we must take into account these tissue differences and, unfortunately, we do not know how \( \beta \)-cell mitochondria would respond. The site of inhibition was believed to be at the level of NAD, although the only evidence is the partial protection exerted by NAD, and this could be explained in other ways. We also know nothing about the permanency of such cycle inhibitions in the \( \beta \)-cells. If the inhibitions described by Younathan are irreversible, the concentrations of alloxan estimated to occur from diabetogenic doses would certainly interfere markedly with the operation of the cycle and would presumably induce damage of the cells. If enzyme inhibition is indeed responsible for the changes in the \( \beta \)-cells, it may well be that no single enzyme can be implicated and that several systems are inactivated simultaneously. These matters will be discussed more fully in a later section (page 412).

**EFFECTS ON METABOLISM**

Alloxan may be thought of as structurally related to part of the riboflavin molecule, and for this reason its effect on respiration was studied by Bernheim (1938). The oxygen uptake of guinea pig liver suspensions in increased and the R. Q. is simultaneously decreased from 0.83 to 0.69 by alloxan at 0.17 mM, this concentration exerting maximal effects. There is an 8- to 10-fold stimulation of ethanol oxidation, with increased for-
mation of acetaldehyde, but no stimulation was observed with other substrates, such as lactate, pyruvate, citrate, succinate, acetate, and others. The acceleration of ethanol oxidation is slight in kidney and absent in brain suspensions. It is likely that this effect is due to the hydrogen-transporting ability of alloxan, but methylene blue had no such effect on ethanol oxidation. Even though alloxan could participate in hydrogen transfer with the other substrates, it probably inhibits some of the cycle enzymes so potently that the stimulation would not be evident. However, other instances of respiratory stimulation have been reported for rat liver homogenates (Klebanoff, 1955), erythrocytes (Robuschi, 1952), and mycobacteria (Müller et al., 1960). Some increased oxygen uptake could be nonenzymic, the result of the oxidation of thiols or ascorbate with subsequent oxidation of dialurate by oxygen. Respiration may also be depressed by alloxan, as in rat diaphragm (Gray and DeLuca, 1955), dog kidney (Villasante and Diaz, 1950), or cornea (Langham, 1953), or apparently unaffected, as has been reported in a number of tissues. Mechanisms by which alloxan can alter respiration are so manifold that simple experiments demonstrating either stimulation or depression are not usually interpretable.

Carbohydrate Metabolism

The uptake of glucose by diaphragm is surprisingly resistant to alloxan, since Haft and Mirsky (1952) found no effect at 10-50 mM, and Gray and DeLuca (1955) observed only a 20-25% depression at 500 mM (in both cases, media kept at pH 3.5 to increase the stability of the alloxan). LeFevre (1948) stated that the uptake of glucose in erythrocytes is inhibited by alloxan but gave no data. The lack of effect with muscle would not be expected in view of the sensitivity of hexokinase to alloxan, but it may be that under the rather abnormal conditions used the uptake was governed by simple diffusion rather than phosphorylation. Both Haft and Mirsky (1952) and Gray and DeLuca (1955) agree that the conversion of glucose to glycogen is blocked by alloxan in diaphragm; indeed, instead of glycogen synthesis, one finds a loss of glycogen (one must remember the very high concentrations of alloxan used in this work). Gluconeogenesis from lactate in liver is well inhibited by alloxan (Carrasco-Formiguera and Mendoza, 1950); this may be an expression of actions on the EM pathway. Glycogenolysis to form glucose in liver slices is not affected by 1.4 mM alloxan (Canzanelli et al., 1946), but in perfused liver both the spontaneous and epinephrine-induced release of glucose is blocked by the injection of 10 mg alloxan into the perfusion fluid (Goldner and Jauregui, 1953). These effects on the liver are believed by some to explain certain phases of the blood glucose curve after administration of alloxan. Gray and DeLuca (1955) have emphasized that vitamin E is antagonistic to the actions of alloxan on carbohydrate metabolism in diaphragm, and it appears that
vitamin E-deficient diaphragm is more susceptible to alloxan with respect to glucose uptake, glycogenesis, and respiration.

We turn now to the effects of alloxan on glycolysis, which one would expect to be quite sensitive on the basis of the results with glycolytic enzymes. Kensler et al. (1942 b) found that alloxan at 0.22 mM is very depressant to the fermentation of fructose-1,6-diP by yeast extracts if the NAD level is low but not if it is high. This could mean that NAD is protecting the SH groups of glyceraldehyde-3-P dehydrogenase, as it is known to do against various SH reagents. Anaerobic glycolysis in frog muscle brei is about 50% reduced by 1.2 mM alloxan and nearly 90% by 2.5 mM (Gemmill, 1947), and aerobic glycolysis of the cornea is depressed 50% by the injection of 2.5 mg alloxan into the anterior chamber (Langham, 1953). However, Villasante and Diaz (1950) detected no inhibition of anaerobic glycolysis in dog kidney. Field et al. (1960) examined the formation of labeled CO₂ from glucose in human β-cell tumor tissue. There seems to be a very active pentose-P shunt, but alloxan was claimed to have no effect on the formation of C¹⁴O₂ from either glucose-1-C¹⁴ or glucose-6-C¹⁴ (there is possibly a slight shift toward the pentose-P pathway)*. In addition to the low concentration of alloxan used, there is the possibility that β-cell tumors are much more resistant to alloxan than normal β-cells (see page 391). It is not known if glycolysis is significantly affected in the tissues of the whole animal, or which is more vulnerable, the EM pathway or the cycle. The elevation of blood keto acids reported by El Hawary (1955) cannot be attributed to the direct action of alloxan since the values were obtained 48–72 hr after the administration and are certainly the result of insulin deficiency.

Lipid Metabolism

Rusch and Kline (1941) studied the effects of various carcinogenic and carcinostatic agents on the oxidation of phospholipids by glutathione or ascorbate. Alloxan inhibits this 28% at 0.35 mM, probably by its oxidation of the catalysts. The only importance of this observation is to demonstrate that alloxan can exert nonenzymic effects on certain oxidation reactions. The inhibition by alloxan of the oxidation of oleate by E. coli reported by Singer and Barron (1945) could be due to an action on the fatty acid helix or the cycle, but one cannot be certain.

The effects of alloxan on lipid synthesis from acetate in rat liver homogenates are marked and quite interesting (see accompanying tabulation) (Seaife and Migicovsky, 1957). The inhibition of the incorporation of acetate

* They state that 0.01 mg alloxan was placed in each flask in a Dubnoff shaker. The volume was not given but if one assumes 10 ml, the alloxan concentration was 0.007 mM, which would hardly be expected to produce much effect.
into cholesterol and fatty acids appears to be due primarily to the interference with acetate activation. The reason for the stimulation of oxygen uptake and the source of the excess CO₂ produced are not well understood.

<table>
<thead>
<tr>
<th>% Change by alloxan at:</th>
<th>0.14 mM</th>
<th>0.42 mM</th>
<th>0.70 mM</th>
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</thead>
<tbody>
<tr>
<td>Acetate → cholesterol</td>
<td>−11</td>
<td>−55</td>
<td>−92</td>
</tr>
<tr>
<td>Acetate → fatty acids</td>
<td>−27</td>
<td>−62</td>
<td>−92</td>
</tr>
<tr>
<td>Acetate → CO₂</td>
<td>−29</td>
<td>−56</td>
<td>−65</td>
</tr>
<tr>
<td>Total CO₂ formed</td>
<td></td>
<td>+112</td>
<td>+152</td>
</tr>
<tr>
<td>O₂ uptake</td>
<td>+11</td>
<td>+196</td>
<td>+281</td>
</tr>
</tbody>
</table>

In the whole animal, the effects on liver lipids are complicated by the early hyperglycemia and possible adrenal activation. When rats are injected with 40 mg/kg alloxan intravenously there are increases of 7% and 63% in total lipids and neutral fat, respectively, at 4 hr, but no changes occur in phospholipids or cholesterol (Dury, 1954). Although these changes were taken as evidence for a hepatotoxic action of alloxan, one would like to have better evidence that these secondary factors are not actually responsible.

**Nucleic Acid Metabolism**

Liver DNA is elevated in alloxan-diabetic rats. Since it was not known whether this was due to the alloxan or to the diabetic state, Bass *et al.* (1953) investigated the problem in greater detail. Alloxan was injected intraperitoneally at a dose of 400 mg/kg and on the fifth day the DNA in the liver had increased from 30.1 to 36.5 mg DNA-P/100 g, the RNA level being unchanged. Pancreatectomy does not alter the liver DNA/RNA ratio, although it to some extent increases both DNA and RNA. However, the DNA per nucleus is not changed by pancreatectomy and is increased by alloxan. It was concluded that the elevation of DNA is the result of some specific direct effect of alloxan and is not related to the diabetic state. It is not known if alloxan can in some manner stimulate DNA biosynthesis, but it would seem unlikely. Harris (1963) showed that alloxan at 1 mM inhibits rather strongly the incorporation of labeled uracil into the uridine nucleotides in rat brain homogenates, and evidence was presented that the major site of action is the phosphorylation of UMP to UDP. This has been confirmed by noting the accumulation of uridine and UMP, and eliminating other possible mechanisms (Younathan *et al.* 1963, 1964). As far as I know, this is the only demonstrated action of alloxan on the formation of nucleotides or nucleic acids.
Protein Synthesis

The complexities which may be encountered with alloxan are well illustrated by the effects on tryptophan peroxidase levels in rat liver (Schor et al., 1958; Schor and Frieden, 1958). It is known that tryptophan and insulin induce the formation of liver tryptophan peroxidase. The administration of alloxan leads to a biphasic curve for the levels of this enzyme: an initial peak around 6 hr (6-fold increase) is followed by a fall to normal levels and then a second rise at 72 hr (8- to 10-fold increase). These fluctuations are independent of blood glucose. The initial peak may be due to the release of insulin or to an adrenal-mediated stress reaction, since adrenalectomy abolishes this early peak, and the delayed peak may be due to tryptophan liberated from cellular breakdown in certain tissues. These effects are discussed here because superficial interpretation or limited experimental data might lead one to conclude that alloxan directly affected protein synthesis. It may, but these results can be explained otherwise. There has been essentially no work indicating what alloxan may do to protein synthesis. Berenbaum (1962) could find no depression of antibody formation by alloxan at high doses in mice given vaccine, but this is not a very good test for protein synthesis. It is interesting to note, in view of the results discussed above, that alloxan inhibits the conversion of tryptophan to kynurenine both in vitro and in vivo (Takagi et al., 1958). It is not known whether the action is on tryptophan pyrrolase or on kynurenine formylase, but it might provide an additional mechanism for the effects of alloxan on tryptophan peroxidase levels, inasmuch as increased tryptophan levels might result from the inhibition.

MECHANISMS INVOLVED IN THE EARLY HYPERGLYCEMIA AND HYPOGLYCEMIA

There have been several postulated mechanisms to explain the early changes in blood glucose following the administration of alloxan, and these have often revolved around the problem of whether the site of action is pancreatic or extrapancreatic. Certainly the major site of action seems now to be on the $\beta$-cells and knowledge of the early changes may provide a clue to the diabetogenic mechanism.

Initial Hyperglycemia

The rapidly developing hyperglycemia might most reasonably be due to a suppression of glucose utilization by the tissues or to a release of glucose from the tissues (particularly the liver) at a rate faster than its utilization. It was suggested originally by Dunn et al. (1943 a) that the hyperglycemia may result from hepatic glucose release due to adrenal discharge
of epinephrine, and this was supported by several reports that adrenalectomy abolishes the hyperglycemic response (Goldner and Gomori, 1944; Kirschbaum et al., 1945; Gaarenstroom and Siderius, 1954) and that sympathetic blockade with ergotoxin does likewise (Corkill et al., 1944). It was also supported by the fact that an epinephrine-like rise in blood pressure occurs after injection of alloxan (Hard and Carr, 1944). Histological changes in the adrenal medulla, including fragmentation and shrinkage of the cells, were observed. The glucose seems to come mainly from the liver since hepatectomy abolishes the hyperglycemia (Houssay et al., 1945), and during the development of diabetes the liver glycogen varies inversely with the blood glucose, being low during the initial hyperglycemia (Cullimore et al., 1953).

Despite the simplicity of this theory and the apparently good evidence for it, this mechanism has been questioned and several experimental results are incompatible with it. (1) Hypophysectomy abolishes the hyperglycemic response and the presence of adrenal cortex seems to be necessary (Kirschbaum et al., 1945). A pituitary-corticoadrenal activation antagonizing the action of insulin was postulated. (2) Administration of DDD* produces atrophy of the adrenal cortex and loss of the hyperglycemic response to alloxan (Nichols and Sheehan, 1952), again pointing to some participation of the adrenal cortex. (3) In rabbits whose β-cells have been destroyed by alloxan, the hyperglycemic response to alloxan is much reduced (Brunfeldt and Iversen, 1950). This points to a pancreatic site of action. (4) Injection of alloxan into the portal vein produces a more marked hyperglycemia than injection into the saphenous vein (Houssay et al., 1945). Furthermore, the authors claimed that adrenalectomy does not abolish the hyperglycemic response, and that the effect is more likely on the liver. The discrepancy in the effects of adrenalectomy is not easy to explain. Lukens (1948) believes that Houssay's data indicate an effect to reduce the hyperglycemia, and, on the other hand, the animals were under chloroform anesthesia, which might have decreased the magnitude of any adrenal discharge. We have discussed the effects of alloxan on the glucose and glycogen metabolism of liver (page 402), and there is very little evidence that alloxan directly causes a release of glucose; indeed, spontaneous and epinephrine-induced glycogenolysis, on the contrary, is usually inhibited.

The hyperglycemia could be due in part to a sudden and complete cessation of insulin secretion and Dixit et al. (1962) found that the insulin level in the pancreas is elevated some 12% 2 hr after alloxan. The observation by Kurita (1955) that Zn** prevents the early hyperglycemic response without altering the eventual diabetic state is very interesting but difficult to understand. It seems impossible to formulate a mechanism which is

* 2,2-Bis(p-chlorophenyl)-1,1-dichloroethane.
not contradicted by some experiments. Increased adrenal and sympathetic activity is probably involved, at least in part, but we do not know the site of alloxan action; it could be a nonspecific stress reaction or directly on the adrenals, the latter being favored by the marked histological changes and the severe fall in adrenal SH groups (page 387). Contributions could be made from suppression of insulin release of a general minor depression of hexokinase activity. If it is true that the degree of initial hyperglycemia is correlated with the severity of the final diabetic state, it seems more likely that the primary action is on the $\beta$-cells.

**Hypoglycemia**

Hypoglycemia usually results from (1) increased utilization of glucose by the tissues, (2) increased renal excretion of glucose, or (3) a suppression of the release of glucose from the tissues into the blood. The renal mechanism can be immediately eliminated for alloxan since there is no elevation of glucose excretion (Wrenshall et al., 1950). It is not so easy to decide definitely between the other two possibilities.

The most commonly accepted mechanism for the hypoglycemia is that insulin, preformed and stored in the $\beta$-cells, is released during the damage to these cells by alloxan (Hughes et al., 1944; Kadota, 1950). The original suggestion by Jacobs (1937) that alloxan itself might have an insulin-like action has been adequately shown to be incorrect, as has the early theory of Dunn et al. (1943 a) that there is a transitory stimulation of the $\beta$-cells by alloxan, since at the time of hypoglycemia these cells are usually quite severely damaged. A very early stimulation, however, may occur and account for the occasional hypoglycemia noted during the first hour but is usually masked by the hyperglycemia (Gaarenstroom and Siderius, 1954). The following evidence for the release of insulin from the degenerating $\beta$-cells as the origin of the hypoglycemia can be cited. (1) Pancreatectomy or previous destruction of the $\beta$-cells with alloxan abolishes the hypoglycemia, so that pancreatic tissue seems to be necessary for this response (Goldner and Gomori, 1944; Kennedy and Lukens, 1944; Ridout et al., 1944). (2) The hypoglycemic response can be simulated by the injection of insulin in the amount occurring within the pancreas (Hughes et al., 1944). (3) The insulin content of the pancreas appears to fall during $\beta$-cell necrosis and at the beginning of the hypoglycemia, but unfortunately no values were obtained during the hypoglycemic stage (Ridout et al., 1944). (4) Alloxan does not alter the utilization of glucose in the eviscerated cat (Corkill et al., 1944). (5) Sulfonlureas stimulate the release of insulin from the pancreas so that pretreatment with these drugs should reduce the hypoglycemic response to alloxan if it is mediated through insulin release, and this has been found to be the case (Klimas and Searle, 1958; Volk and Lazarus, 1961). (6) Neither adrenalectomy nor hypophysectomy prevents
the hypoglycemic response to alloxan and so these tissues are not involved (Goldner and Gomori, 1944; Kirschbaum et al., 1945). These results point rather strongly to insulin release being the primary factor in the hypoglycemia.

There are some results which indicate that this may not be the entire story. The rise in liver glycogen during the hypoglycemic phase is not typical of an insulin effect, since insulin does not promote glycogen storage in the liver; indeed, hepatic glycogen usually falls secondary to the hypoglycemia (Cullimore et al., 1953). Wrenshall et al. (1950) did not find a decrease in pancreatic insulin at 9 hr, but postulated that insulin synthesis might be accelerated to compensate for the loss. The recent results of Dixit et al. (1962) also do not indicate a fall in pancreatic insulin before 24 hr, although after that it disappears rapidly. Determinations were made only at 2 hr and 24 hr and not during the hypoglycemic phase, but it would be surprising if a significant fall occurs between these times. Increased synthesis of insulin might occur but it seems unlikely in view of the state of the cells during this period. We have seen that alloxan rather readily inhibits glycogenolysis in the liver (Goldner and Jauregui, 1953), but whether the concentrations used are comparable to those occurring in vivo is not known. Furthermore, this effect is immediate and the hypoglycemia occurs several hours later. The degree of liver damage was thought to parallel the degree of hypoglycemia (Ramfjord, 1952), but the doses were quite high and the work was done in monkeys, which are not so specifically responsive to alloxan as other species. Houssay et al. (1945) concluded that the hypoglycemia cannot be due to insulin release because it occurs in acutely depancreatized dogs; if the alloxan is given 24–48 hr after pancreatectomy, no hypoglycemia is seen. The explanation for these results is not evident. Another surprising observation is that 23–58 days after ligation of the pancreatic duct the total blood glucose response to alloxan is reduced and, inasmuch as such ligation produces parenchymal atrophy with little obvious changes in the islets, it might indicate that the parenchyma is somehow involved (Walpole and Innes, 1946). It was thought that the ligation might also cause some ischemia of the islets so that the alloxan could not reach them, but this does not sound very convincing. Most of this evidence is rather circumstantial and probably does not invalidate the theory of insulin release, but taken all in all it indicates that the situation is more complex and that some additional factors may be involved.

**MECHANISM OF THE DIABETOGENIC ACTION**

The problem here is not to explain the late and permanent hyperglycemia, since this is unquestionably due to a deficiency of insulin, but to inquire into the mechanisms by which alloxan causes a selective β-cell necrosis.
Let us summarize a few of the known characteristics of alloxan action. 
(1) Alloxan produces in most animals at a proper dose a selective necrosis of the pancreatic \(\beta\)-cells so that after several days insulin secretion no longer occurs. (2) Before the final diabetic state develops, alloxan brings about variations in the blood glucose by mechanisms which are partly, or perhaps entirely, pancreatic. (3) These early changes in blood glucose are not in themselves the cause of the diabetic state, since counteracting these changes does not modify the eventual destruction of the \(\beta\)-cells. (4) There is an early and temporary loss of SH groups in various tissues, including the pancreas. (5) The diabetogenic action of alloxan can be antagonized by the administration of glucose, nicotinamide, and \(\text{Zn}^{++}\), as well as by numerous substances reacting with alloxan. (6) Alloxan is very unstable and it is believed that the effects on the \(\beta\)-cells must be exerted within a few minutes of an intravenous injection. (7) The action on the \(\beta\)-cells is probably exerted by alloxan itself rather than any of its breakdown products or metabolites; at least no substance formed from alloxan is diabetogenic with the exception of those readily converted back to alloxan. It may also be convenient to summarize here the evidence for the important conclusion that alloxan exerts its effect within several minutes. First, alloxan is very unstable in the blood and is almost entirely destroyed within 5 min. Second, antagonists must be given either before or within 2–3 min of the alloxan injection to be effective. Third, cytological changes in the \(\beta\)-cells can be seen within 5 min. Finally, ligation of the blood vessels supplying part of the pancreas for 2 min following an alloxan injection protects that portion of the pancreas (Gomori and Goldner, 1945).

Before discussing the theories of the diabetogenic action, we must try to determine if the action is indeed directly on the \(\beta\)-cells. One possibility is a specific spasm of the arterioles supplying the islets, as suggested by Poulsen (1946). It is true that alloxan has some vasoconstrictive activity (Macqueen, 1952). Blanching of the region supplied by an injected artery was observed and a transient rise was noted in the blood pressure, which is not abolished by adrenalectomy, nephrectomy, hypophysectomy, decerebration, hexamethonium, or dibenamine. It appears, however, that this explanation is untenable. (1) The \(\alpha\)-cells are also supplied by the same arterioles as the \(\beta\)-cells and yet show no necrosis, although this might imply a differential sensitivity to anoxia, which is no harder to imagine than a differential sensitivity to alloxan. (2) There is no evidence that such a very selective vasospasm can occur, and it is certainly more difficult to imagine such a selectivity. (3) Epinephrine prevents the diabetogenic action, presumably by a vasoconstriction so that alloxan cannot reach the \(\beta\)-cells, and yet no damage to the \(\beta\)-cells occurs from epinephrine. (4) Ligation of blood vessels supplying the pancreas for 1–6 min does not lead to damage to the \(\beta\)-cells, and this appears to be conclusive evidence
against the vasoconstrictive theory. We may thus assume that the action is on the \( \beta \)-cells.

**Possible Reasons for the Selective Damage to the \( \beta \)-Cells**

There are two important questions to be answered. Why are the \( \beta \)-cells so remarkably susceptible to alloxan, and what is the mechanism by which alloxan damages the \( \beta \)-cells? The first question will be considered in this section. Whatever the reason for the high susceptibility of the \( \beta \)-cells, it is evident that this is a common property of insulin-secreting cells, inasmuch as it occurs in so many types of animal.

(A) **Selective accumulation of alloxan in the \( \beta \)-cells.** Total analyses of the pancreas and radioautography of the islets have failed to demonstrate accumulation of alloxan; indeed, there seems to be generally a lower concentration than in many other tissues (page 394). There is thus sufficient evidence to eliminate this unlikely hypothesis.

(B) **The intracellular pH of the \( \beta \)-cells is low.** Alloxan is more stable the lower the pH and it has been suggested that the \( \beta \)-cells may have a particularly low pH, the alloxan level thus remaining higher for a longer period (Klebanoff and Greenbaum, 1954). It is doubtful if the alkaline acinar secretion would affect the intracellular pH of the \( \beta \)-cells, as postulated. No reliable pH determinations in these cells have been made so there is at present no positive evidence for this hypothesis.

(C) **The extra- or intracellular glucose concentration of the \( \beta \)-cells is low.** Glucose protects the \( \beta \)-cells against alloxan and we have seen that Artteta et al. (1954) thought the glucose concentration around these cells would determine their susceptibility to alloxan. Bhattacharya (1953) believed the \( \beta \)-cells might contain low concentrations of glucose because of the high insulin content. It seems very unlikely that the glucose concentration around the \( \beta \)-cells would be different from that in other tissues in view of the rapid equilibration of glucose between plasma and intercellular tissue fluid, and the fact that administration of glucose protects the cells. Little can be said about the internal glucose until direct determinations are made.

(D) **The \( \beta \)-cells contain a low concentration of glutathione.** Glutathione readily reacts with alloxan and exerts a protective action. Using 2,2'-dihydroxy-6,6'-dianaphthyldisulfide, a reagent measuring protein SH groups, Barrnett and Seligman (1952) found that islet tissue is very low in such SH groups, and Lazarow (1954 a) postulated that the glutathione level may also be low, due to the utilization of much of the cysteine in insulin synthesis. Lazarow felt that a low concentration of glutathione might predispose the \( \beta \)-cells to alloxan damage, other cells being more adequately protected. Inasmuch as alloxan is so unstable in solutions free of thiols,
it is doubtful if high or low levels of glutathione would make much difference. Furthermore, MacDonald (1959) found no significant difference in the SH group contents of acinar and islet tissue, using Bennett's red SH reagent.

(E) *The reducing power of β-cells is relatively low.* Alloxan is readily reduced to dialurate by various substances and certain dehydrogenase systems in the cell. If the reducing activity of the β-cells were low, less alloxan would be converted to dialurate and would be free to act for a longer time. Supravital staining of the pancreas with Janus green B and placing the tissue under anaerobic conditions lead to bleaching of the dye in those regions with the highest reducing activity, and Bensley (1911) had shown that the bleaching rate in the islets is very slow. Since Janus green B is reduced by NADH-flavoprotein systems and oxidized by the cytochromes, the state of the dye roughly indicates the balance between oxidative and reductive potencies in a cell. The results on islet tissue point to a low concentration of dehydrogenases and their coenzymes. Lazarow and Cooperstein (1951) took advantage of the separable islets of the toadfish and demonstrated a relatively low level of succinate dehydrogenase. The ratio of the dehydrogenase to cytochrome oxidase is low and if this is true of other dehydrogenases, the alloxan ≥ dialurate equilibrium would be shifted to the left, i.e., any dialurate formed would be rapidly reoxidized to alloxan. Succinate dehydrogenase has also been shown to be lower in islet tissue than in acinar tissue by the neotetrazolium technique (Barrnett, 1951). Lazarow and Cooperstein thus suggested that the effective level of alloxan would remain higher for a longer time in islet tissue, and that this might account for the selective action. It must be mentioned that certain other tissues, e.g., the gonads, exhibit a similar reducing deficiency and are not susceptible to alloxan. One again wonders if this effect could be sufficiently great to prolong the alloxan concentration significantly, inasmuch as other pathways of alloxan inactivation are quite active.

(F) *The β-cells contain a low concentration of some vital enzyme or cofactor.* The effects of pseudoirreversible or titration inhibitors depend on the relative amounts of the component attacked in the different tissues (page I-78). Ackermann and Potter (1949) suggested that the selective damage to the β-cells by alloxan might be an example of this, the β-cells containing a smaller amount of some SH component with which alloxan reacts. Lazarow (1954 a) has also considered the possibility that the β-cells contain a low level of some thiol (such as glutathione or coenzyme A), the inactivation of which would impair glyceraldehyde-3-P dehydrogenase or certain transacetylations, or of some SH enzyme with which alloxan readily reacts. It is, of course, not only a matter of the level of some critical substance,
but the degree of dependence of the tissue on that substance. Until we know more of the nature of such a critical component in the $\beta$-cells, it is difficult to evaluate this theory of selectivity.

It is thus clear that despite the many postulates which have been made, we do not understand at all the reason for the greater susceptibility of the $\beta$-cells. It is difficult to explain satisfactorily why no other SH reagents act in this way if SH components of the $\beta$-cells are the targets for alloxan action. Perhaps insufficient attention has been paid to the one definite characteristic of $\beta$-cells, their special function of synthesizing and releasing insulin, especially in response to an elevated glucose concentration. Unfortunately, very little is known of the mechanisms and controls for the secretion of insulin.

Possible Mechanisms by Which Alloxan Damages the $\beta$-Cells

One can only speculate as to how alloxan acts on the $\beta$-cells. With respect to the possible sites of the action, most of the theories can be classified as follows: (1) an SH enzyme, (2) a thiol cofactor (e. g., glutathione or coenzyme A), (3) a metal ion or metalloenzyme, (4) an enzyme catalyzing some reaction in pyrimidine or purine metabolism, or (5) a nonenzymic membrane component. One general difficulty is understanding how the reaction of alloxan with these sites so rapidly and extensively damages the $\beta$-cells. A simple inhibition of some metabolic pathway seldom evokes such immediate cytological changes in tissue, and many inhibitors capable of blocking the major pathways do not produce alloxan-like effects in the $\beta$-cells. Even anoxia for several minutes does not harm these cells. This, taken in conjunction with the selectivity, must mean that some component intimately related to the maintenance of cell structure is attacked, or some component involved in the particular functions of the $\beta$-cells. The various theories will be briefly mentioned and discussed.

(A) Inhibition of hexokinase. Griffiths (1949) thought that previous indirect evidence might point to an inhibition of hexokinase, and this received some support from the observation by Bhattacharya (1954) that glucose can protect the $\beta$-cells against alloxan. The main difficulty is that the protective potencies do not run parallel with the affinities of the known hexokinases for various sugars (Villar-Palasi et al., 1957), but one must admit that the properties of $\beta$-cell hexokinase are unknown. The sensitivities of the examined hexokinases to alloxan are not enough to allow one to predict a very significant inhibition at the concentrations reached in vivo, and unless a very marked block occurs it is difficult to understand how rapid structural changes would be produced. Finally, unless the $\beta$-cell hexokinase is much different from that of other tissues,
it is hard to account for the selective action. There is today very little evidence in favor of this theory.

(B) Inhibition of enzymes other than hexokinase. Very few enzymes markedly susceptible to alloxan have been found, as discussed earlier (page 400). Some of the cycle enzymes are certainly inhibited at low concentrations (Younathan, 1962) but if this is the site of action in the \( \beta \)-cells, some further assumptions must be made to account for the selective damage to these cells. Since the mitochondria used in this work were from kidney, one might expect on this basis a rather marked renal toxicity, whereas only doses higher than the diabetogenic seem to affect the kidney. Burgan and Lorch (1947) attempted to correlate diabetogenic activity with the inhibition of alkaline phosphatase, but the fact remains that no inhibition of this enzyme could be demonstrated \textit{in vivo}. The inhibition of UMP kinase demonstrated by Harris (1963) could hardly account for the initial \( \beta \)-cell damage, nor could inhibition of acetylating systems as reported by Cooperstein and Lazarow (1958). The malate dehydrogenase of islet tissue is unaffected by diabetogenic doses of alloxan up to 12 hr, but a steady loss of activity is then observed, probably due to secondary changes resulting from necrosis (Dixit \textit{et al.}, 1963). Although malate dehydrogenase is about 3 times more active in the islets than in acinar tissue, it is certainly not involved in the alloxan action. The most interesting approach of this type has been by Lazarus \textit{et al.} (1962). They studied histochemically the effects of diabetogenic doses of alloxan on several enzymes in the \( \beta \)-cells. No early changes were noted for succinate dehydrogenase, cytochrome oxidase, glucose-6-P dehydrogenase, NADP diaphorase, glucose-6-phosphatase, or acid phosphatase; during the later necrotic stages, of course, the enzyme activities fall markedly. The one enzyme tested showing a rapid inactivation is the extramitochondrial ATPase. It was stated that cortisone administration causes this enzyme to disappear from the \( \beta \)-cells and simultaneously there is an increase in the resistance to alloxan, which is strange since, if the ATPase is of such importance to the cells, one would expect its disappearance to be detrimental. Possibly cortisone in some manner alters the \( \beta \)-cell metabolism so that this enzyme is not necessary. If this ATPase were located in the \( \beta \)-cell membrane, its inhibition might have disastrous effects on the membrane structure. There is need for a thorough investigation of various ATPases \textit{in vitro} and possibly this could be done using toadfish or sculpin islets.

(C) Inactivation of glutathione. For some years Lazarow (1954 a) emphasized the importance of glutathione in the diabetogenic action of alloxan, inasmuch as the reaction between them occurs so readily, and it was quite easy to imagine how glutathione might be of importance in the \( \beta \)-cells, particularly relative to certain enzymes such as 3-phosphoglyceraldehyde
dehydrogenase. This theory is not so popular today because it is difficult to understand why other SH reagents, some of which reduce pancreatic glutathione (Hultquist, 1958), do not share with alloxan a selective effect on the β-cells. Also it is not clear how inactivation of glutathione could produce such rapid damage.

(D) Inactivation of coenzyme A. More recently Cooperstein and Lazarow (1954, 1958) found a rather potent inhibition of a liver acetylating system, part of this being due to inactivation of coenzyme A. If coenzyme A is inactivated in the β-cells, it would seriously interfere with α-keto acid oxidations and the operation of the cycle, but how this could be selective or responsible for rapid cytological changes (since the β-cells are resistant to short periods of anoxia) is not understood.

(E) Inhibition of metalloenzymes. Since alloxan chelates with certain metal ions, and Zn++ is in some manner involved in islet function, it was natural to postulate that alloxan might act on the β-cells by either removing Zn++ or inactivating a Zn++-dependent enzyme. Furthermore, the only other substances known to produce a similar selective destruction of the β-cells, accompanied by a typical three-phase blood glucose variation, are 8-hydroxyquinoline (oxine) and diphenylthiocarbazone (dithizon), both effective chelators of Zn++ and other heavy metal ions (Kadota, 1950; Kadota and Midorikawa, 1951). If Zn++ (0.1 millimole/kg) is injected immediately before a diabetogenic dose (0.28 millimole/kg) of alloxan in rats, the incidence of diabetes is appreciably reduced (Lazarow and Patterson, 1951). Fe++ is as effective as Zn++, and Co++ is some 5 times more effective. It seems unlikely that the metal ions simply react directly with the alloxan because there is insufficient metal ion (in the case of Co++ the alloxan is injected in 15-fold excess), unless the metal ions act catalytically to inactivate the alloxan. Kurita (1955) could detect no protection by Zn++ against the diabetogenic action in rabbits, but this may be due to technical differences or the fact that a higher alloxan dose is required in rabbits. The injection of alloxan in dogs, rabbits, and rats leads to a marked rise in serum Zn++ (more than doubled) and urinary Zn++ (15-fold increase) at around 3 hr (Maske et al., 1952). This might on the surface be interpreted as good evidence for the ability of alloxan to interfere with Zn++ balance, but it appears that the Zn++ actually arises from hemolyzed erythrocytes, since α-tocopherol, which protects against hemolysis but not against islet damage, reduces or abolishes the rise in serum and urinary Zn++. Histochecmical determination of Zn++ in the islets showed no change in Zn++ content 15 min after the injection of alloxan, although later there is a definite loss of Zn++ from the islets, this perhaps being associated with the necrosis. A complication making it difficult to study the effects of alloxan on Zn++ function and distribution is the fact that alloxanate prob-
ably chelates Zn$$^{++}$$ and other ions much better than alloxan (page 378) (Resnik and Cecil, 1956). Unfortunately, there has been no thorough investigation of the effects of alloxan on the metalloenzymes so that we do not know if alloxan can complex with a protein-bound metal ion.

(F) Reaction with $$\beta$$-cell membranes. The rapid and irreversible cell damage seen with alloxan might be more readily interpreted as an effect on the $$\beta$$-cell membranes whereby their permeability or integrity is altered. Dixon et al. (1960 b) observed that alloxan causes an early separation of the $$\beta$$-cells from each other and the surrounding capillaries, and suggested that alloxan might react with a layer of protein on the surface of the cells. Watkins et al. (1961) studied the permeability of toadfish islet cells to mannitol-C$$^{14}$$ as a measure of the integrity of the plasma membrane, since mannitol normally does not penetrate rapidly. Alloxan at 0.25 mM was found to double the rate of mannitol penetration. Alloxanate is inactive. The permeability of kidney, heart, brain, gill, muscle, and liver cells is not altered by alloxan. These data are thus consistent with the idea that alloxan selectively alters the $$\beta$$-cell membrane. The membrane component with which alloxan acts is, of course, unknown, but it might be involved in the release of insulin since the $$\beta$$-cells are specialized for this function.

(G) Release of trypsin from the exocrine tissue. Ligation of the pancreatic duct leads to atrophy of the pancreatic parenchyma without affecting the islets, and simultaneously there is a reduction in the susceptibility to alloxan (page 408) (Walpole and Innes, 1946). This might imply that the acinar tissue is involved in the diabetogenic action, perhaps by causing the release of trypsin which damages the $$\beta$$-cells. However, trypsin injections do not disturb the $$\beta$$-cells. Urinary diastase excretion is essentially abolished by alloxan and this might also be interpreted as an effect on the acinar tissue (Bernhard et al., 1947). Ethionine administration for long periods produces resistance to alloxan and causes a fibrosclerosis of the exocrine tissue (Gambassi and Del Gatto, 1956). Pancreatic duct ligation leading to parenchymal atrophy does not prevent the development of diabetes from alloxan in rats, but prevents it in rabbits although the $$\beta$$-cells are damaged, so that it was concluded that trypsin release is not an important factor (Ries and Allegretti, 1957). It is difficult to understand how the $$\beta$$-cells could be eliminated in rabbits without the appearance of the diabetic state. One notes also that injury to the $$\beta$$-cells is not observed in vitro, even at high concentrations of alloxan (Becker et al., 1962). The significance of these observations cannot be evaluated at this time.

Progress in this field must await more detailed investigations of the metabolic and functional peculiarities of the $$\beta$$-cells. This has been started in the work of Humbel and Renold (1963) on the glucose and amino acid metabolism of cultured toadfish islet tissue, wherein they showed that
a pentose-P pathway exists, glucose is readily oxidized and incorporated into lipid, and leucine is incorporated into insulin. Studies of changes induced by subdiabetogenic doses of alloxan might also be valuable. Mollerander and Kirschbaum (1949) reported, for example, that rats given 20 mg/kg alloxan intravenously every other day demonstrate a functional impairment of the β-cells without visible changes. One would like to know how alloxan affects the synthesis and release of insulin, and especially if it modifies the response to elevated glucose. Increasing the glucose concentration in the perfusate of both intact and isolated pancreas evokes an augmented output of insulin, and also protects against alloxan. It will be recalled that β-cell tumors are resistant to alloxan, and that obese hyperglycemic mice, with hyperplastic and hypersecreting islets, exhibit only a fall in blood glucose when alloxan is administered (page 391). The β-cells of treated obese mice are heavily granulated while those of treated normal mice are degranulated, as if some effect on insulin synthesis or release has occurred. If the β-cells of rabbits are stimulated by giving cortisone, the response to alloxan is altered, some resistance being manifest and the degeneration of the β-cells assuming a different course (Volk and Lazarus, 1961). All of these results point vaguely to a relationship between the action of alloxan and the controlling factors in insulin synthesis or release. Since the β-cells are specialized to provide insulin, possibly anything which sufficiently disturbs this process could readily and rapidly damage the cells.

Glucose may accelerate insulin liberation from the β-cells by reacting with some receptor in the membrane and alloxan might also attack this site, which would account for the protection afforded by glucose. The protection is presumably not related to the metabolism of glucose since 3-methyl-D-glucose is also effective. The protection observed with barbiturate might also indicate a competition for some site having affinity for structures of this type. Could alloxan by reacting with this site stimulate the formation of insulin to such a degree that the β-cells are damaged, as gastric parietal cells can be damaged by overstimulation of acid secretion? One recalls that the insulin content of the pancreas rises initially after alloxan, and at 24 hr when the diabetic state is developing, the insulin level is not significantly different from normal values (Dixit et al., 1962), and yet there is evidence that during the first 24 hr there is at times a marked release of insulin. Effects of alloxan on the release mechanisms or the glucose control of release might also be invoked. Glucose infused into rats for several hours stimulates the β-cells and depletes them of granules and Zn++; alloxan is now ineffective in inducing the diabetic state, but the susceptibility returns after 1 hr (Kaneko and Logothetopoulos, 1963). The granulation, Zn++ content, and insulin-secreting activity are not the important factors here since β-cells depleted of granules and Zn++ by pro-
longed treatment with insulin are normally susceptible to the cytotoxic action of alloxan. We know too little about all these processes to be able to speculate intelligently as to the mechanisms involved.

**SOME EFFECTS ON TISSUES OTHER THAN THE PANCREAS**

The responses of nerve and muscle to alloxan have been studied very little, but it is certain that no obvious changes occur following diabetogenic doses. Labes and Freisburger (1930) observed convulsions and paralysis in frogs given large doses (300–1250 mg/kg) and felt that the paralysis is central since the muscle are directly excitable. However, an effect on acetylcholine synthesis at the neuromuscular junction is also likely, since Torda and Wolff (1946 b) found a depression of the contraction of the frog gastrocnemius stimulated through its nerve, but no depression of the response of the muscle to acetylcholine, and an inhibition of acetylcholine formation by a brain preparation was demonstrated. Labes and Freisburger (1930) noted that the muscles become stiff and one wonders if this is a Lundsgaard effect. Cardiac standstill occurs both *in vivo* and *in vitro*, but the doses and concentrations required are high and it seems that the heart is quite resistant to alloxan. We have mentioned that alloxan often stimulates smooth muscle and that vasoconstriction, with a rise in the blood pressure, is seen, although released epinephrine may be partially responsible (Macqueen, 1952). High concentrations of alloxan may cause vasodilatation, hyperemia, and congestion, with developing edema, and this is a common response to all SH reagents. Intravenous injections of alloxan in dogs increase the pulmonary arterial pressure due to an increased resistance in the pulmonary vascular bed (Gruhzit et al., 1951). It was postulated that although the pulmonary edema is not caused by the rise in pressure, they may both be the result of capillary damage in the lungs. This has been thoroughly investigated by Aviado and Schmidt (1957), who showed the pulmonary hypertension to be accompanied by a marked vasoconstriction, followed by constriction of the pulmonary veins and lung congestion, this being the cause of the edema.

Since the early work of Dunn *et al.* (1943 b) it has been observed that renal changes can occur at doses above the diabetogenic level, the animals dying in a uremic syndrome (Goldner and Gomori, 1943). The renal lesions were said to be much like those produced by the mercurials, but there seems to be rather marked effects on the glomeruli (Díaz *et al*., 1948; Test *et al*., 1951; Ramfjord, 1952). Indeed, the primary effect may be glomerular. The nephrotoxicity would probably be much more evident if alloxan were not so unstable.

Administration of alloxan causes hemolysis, hemoglobinemia, hemoglobinuria (Kennedy and Lukens, 1944; György and Rose, 1949), and a
fall in erythrocyte count and hemoglobin (Merlini, 1951; Kaito et al., 1954). Progressive hemolysis in vitro is produced by 0.21–56 mM alloxan, with probable formation of methemoglobin above 1.2 mM (Robuschi, 1948). Tocopherol antagonizes the hemolytic action in vivo (György and Rose, 1949). Erythrocytes from vitamin E-deficient animals are lysed much more readily than cells from normal animals. Dialurate or a product formed from dialurate, rather than alloxan, was thought to be the hemolytic agent. Maske and Wolff (1953) believe that Zn$$^{++}$$ is possibly involved in both the hemolysis and the damage by alloxan to the β-cells, and that the mechanisms involved in these two apparently different actions may be similar. Leucocytic phagocytosis is quite potently depressed by alloxan, effects being observed at concentrations as low as 0.035 mM (Köhler et al., 1951).

**EFFECTS ON CELL GROWTH AND PROLIFERATION**

Any effects observed on growth must be exerted during the initial several minutes of contact with alloxan. Hence, presumably only irreversible changes produced in the cells, i.e., those manifesting themselves by a suppression of growth later, would be detected. Most culture media for in vitro study contain numerous substances capable of reacting with alloxan and protecting the cells. Generally speaking, alloxan has not proved to be a very effective growth inhibitor. Cultures of human gingival fibroblasts are not affected by 1.4 mM alloxan during 4 days of observation (Shafer, 1961). The growth of E. coli is not inhibited by 0.7 mM alloxan (Zamenhof and Griboff, 1954). Alloxan can inactivate encephalomyocarditis virus in vitro but has no effect on the proliferation in vivo (Weinstein et al., 1957).

Alloxan and its metal chelates are not antitubercular in mice (Foye et al., 1959). Indeed, the only positive results reported on microorganisms seem to be those of Laskowski et al. (1951, 1954), who found that alloxan in subdiabetogenic doses is very effective in preventing the development of bartonellosis in mice and rats, being as successful as the arsenicals.

The early embryonic development of *Triton alpestris* is slowed by 7 mM alloxan, neurulation being abnormal and the neural plate asymmetrical (Gruber, 1962). The mitotic frequency is reduced, there are chromosomal abnormalities, and spindle formation appears to be disturbed. The most actively proliferating and differentiating cells, as in the dorsal lip, are most affected, and a high proportion become necrotic. Alloxan was classified as a spindle-toxic agent.

The anemia discussed in the previous section is initially due to hemolysis, but there is also a depression of hematopoiesis for several days, as shown by a decline in nucleated erythrocytes in the myelogram, a definite granulocytosis, and an elevation in lymphocytes (Baiardi, 1955). Al-
loxan seems unlikely to be an effective carcinostatic agent, due to its instability, but there is little information on this point. Grobon (1957) has reported that intramuscular injections twice a week of alloxan (20–40 mg) suppress the metastatic spread of endodermic and ectodermic carcinomas, but one must be sceptical of such results because of the difficulty of evaluating metastatic changes. Finally, it may be noted that alloxan, in contrast to most other SH reagents, does not produce blebbing of sarcoma ascites cells (Belkin and Hardy, 1961). Most instances of definite inhibition of cell proliferation thus involve the use of fairly high concentrations of alloxan, and in no case has alloxan been shown directly to be an effective antimitotic agent.
The quinones form a group of highly reactive and occasionally quite specific metabolic inhibitors which present many interesting possibilities for development as tools in enzyme research and as useful drugs in the control of disease. It is a heterogeneous group, embracing substances with several distinct mechanisms of action, of which the reaction with SH groups is only one. The quinones are possibly the most difficult inhibitors to discuss because of this multiplicity of actions. The situation has recently been made more complex with the demonstration of the participation of various types of quinone in electron transport and oxidative phosphorylation, and the realization that exogeneous quinones may disturb these processes by substituting for, displacing, or in other ways interfering with these processes. It is for this reason that quinones are so frequently found to stimulate cell metabolism and functions, the observed effect often representing a balance between the stimulatory and inhibitory actions. The quinones have long been known as potent inhibitors of the growth of microorganisms, and their abilities to induce nuclear and mitotic abnormalities have been investigated more recently. Because of the many types of quinone, natural and synthetic, they represent an ideal field in which to correlate structure with action and in which to determine the effects of group substitution or deletion. Many problems present themselves here for in no case has a potent biological action of a quinone been correlated with a specific mechanism, and it is likely that solution of these problems would provide useful information on the processes of mitosis and growth.

The simplest quinone and, at least with respect to reaction with SH groups, the type substance of this class, p-benzoquinone, was first described and synthesized by Woskresensky in 1838, but serious study of its effects on biological material was delayed. It is true that Woskresensky remarked that it was irritating to the eyes, while Wöhler in 1844 confirmed this irritant effect on mucous membranes. Nevertheless, Wöhler and Frerichs (1848) found that 0.5 g fed to a dog produced no observable effects and that no quinone could be detected in the urine, posing the problem of its fate in the body. Sporadic reports describing miscellaneous effects on animals ap-
peared: e. g., the work of Brieger (1880) on rabbits, of Gibbs and Hare (1890) on dogs, of Schulz (1892) and Baglioni (1905) on frogs, and of Danilewski (1895) on various invertebrates. Much of this early work is well summarized by Ellinger (1923). Certain actions on the nervous system, the heart, and the blood were made evident by this work, but the results were not of sufficient interest to stimulate further more intensive study. Meanwhile some pertinent chemical reactions of the quinones were noted, especially the combination with amines and the important observations by Würster (1887, 1888) that quinones react with amino acids to give colored products, and by Troeger and Eggert (1896) that reactions with thiols often occur readily.

The first significant study of the biological effects of the quinones was made by Thalhimer and Palmer (1911) in Virginia. They showed $p$-benzoquinone to be a very potent bactericidal substance with a phenol coefficient of 160 against *Salmonella typhosa*. Early workers had indeed observed that protein solutions do not spoil in the presence of quinones and concluded that they must be disinfectants, and several had reported that polyhydroxy phenols often show an increased antibacterial activity under conditions favorable for oxidation, a fact soon postulated by Cooper (1913) as due to the formation of quinones. Morgan and Cooper (1924) made some fundamental comparisons of various quinones with respect to their antibacterial actions, but found the presence of biological fluids to depress the activity markedly and concluded that the quinones would be worthless as practical disinfectants. With this proclamation further work stopped and not a single paper was published on this subject until the observations of Raistrick in the early 1940's that several fungal quinones are bacteriostatic. The interest in penicillin and the possibility of the use of some of the fungal quinones as antibiotics led to an intensive period of research culminating in the isolation and synthesis of numerous highly active compounds, and a survey of their actions on metabolic systems. One of the most thorough studies was that of Fieser and his associates during the war on many 2-hydroxy-3-alkyl-1,4-naphthoquinones active against the malarial parasite.

Knowledge of the actions of the quinones on enzymes and metabolic systems is fairly recent despite the fact that it was known for a very long time that proteins, especially those of wool, are readily reacted, and that the mechanism of the antibacterial action had actually been attributed to an inhibition of enzymes by Cooper (1913). Several observations between 1912 and 1928 showed that quinones can often act as hydrogen acceptors in tissues and can function in electron transport in connection with certain enzymes, e. g., xanthine oxidase (Dixon, 1926), but the first definite instance of inhibition was the demonstration by Harvey (1929) that bacterial luminescence is very sensitive to several quinones. The initial work
on enzymes was by Quastel (1933 b) on urease and by Bersin and Logemann (1933) on papain. Metabolic studies were largely ignored until 10 years later and quantitative and thorough investigations on respiration, glycolysis, the cycle, and other important pathways of metabolism are relatively uncommon even today.*

The term *quinones* will refer generically to all the quinones whatever the number of rings or the substituents, *diphenol* will designate any aromatic compound with two hydroxy groups, and *polyphenol* any aromatic compound with two or more hydroxy groups on the rings. The system of abbreviations to be used in the tables is summarized in the following tabulation.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Oxidized form</th>
<th>Reduced form</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Benzquinone</td>
<td>p-Q</td>
<td>p-QH₂ (hydroquinone)</td>
</tr>
<tr>
<td>o-Benzquinone</td>
<td>o-Q</td>
<td>o-QH₂ (catechol)</td>
</tr>
<tr>
<td>Toluquinone</td>
<td>TQ</td>
<td>TQH₂</td>
</tr>
<tr>
<td>p-Xyloquinone</td>
<td>p-XQ</td>
<td>p-XQH₂</td>
</tr>
<tr>
<td>Duroquinone</td>
<td>DQ</td>
<td>DQH₂</td>
</tr>
<tr>
<td>1,2-Naphthoquinone</td>
<td>1,2-NQ</td>
<td>1,2-NQH₂</td>
</tr>
<tr>
<td>1,4-Naphthoquinone</td>
<td>1,4-NQ</td>
<td>1,4-NQH₂</td>
</tr>
<tr>
<td>Menadione</td>
<td>MD</td>
<td>MDH₂ (menadiol)</td>
</tr>
<tr>
<td>9,10-Anthraquinone</td>
<td>9,10-AQ</td>
<td>9,10-AQH₂</td>
</tr>
<tr>
<td>9,10-Phenantheraquinone</td>
<td>9,10-PAQ</td>
<td>9,10-PAQH₂</td>
</tr>
</tbody>
</table>

**CHEMICAL PROPERTIES**

Some of the quinones most commonly used in inhibition studies are shown in the accompanying formulas (other naturally occurring quinones are shown on pages 471, 513).

* Although it is difficult, and perhaps artificial, to separate the groups of quinones for special discussion, the antimalarial naphthoquinones typified by SN-5949, which potently block electron transport at specific sites, will not be treated in this chapter but will be discussed in a future volume with antimycin A and other inhibitors having similar actions.
5. QUINONES

$p$-Benzoquinone

$o$-Benzoquinone

Toluquinone

$p$-Xyloquinone

C umoquinone

Duroquinone

Thymoquinone

5-Methoxy-toluquinone

Tetrachloroquinone (chloranil)

1,2-Naphthoquinone

1,2-Naphthoquinone-4-sulfonate

1,4-Naphthoquinone
CHEMICAL PROPERTIES

Menadione

Menadiol diphosphate

Phthiocol

Lawsone

Juglone

Naphthazarin

2,3-Dichloro-1,4-naphthoquinone

Lapachol

Lomatiol

9,10-Anthraquinone
Molecular Structure

*p*-Benzoquinone does not exhibit aromatic characteristics, inasmuch as the benzenoid resonance in the ring is impossible, and this is evident in the definite distortion of the ring from a regular hexagon, as shown in the crystallographic X-ray diffraction study of Robertson (1935) (structure I). The C=O bond length was found to be appreciably less than expected for a pure double bond (1.22–1.24 Å) and this was attributed to resonance with the ring double bonds, but the electron diffraction study of vaporized *p*-benzoquinone (structure II) by Swingle (1954) gave a more normal length, which is also consistent with the results obtained on similar molecules and the relatively low resonance energy (3–4 kcal/mole).

The surprising observation by Hassel and Naeshagen (1930) that *p*-benzoquinone, usually assumed to be symmetrical and planar, has a dipole moment of around 0.67 debye has been confirmed several times in different solvents (Hammick et al., 1935) and in the vapor phase (Coop and Sutton, 1938). The earlier theories of distortion due to thermal impacts or vibration of the C=O group in the plane of the ring have been shown to be incorrect (Kofod, 1953), and it is now generally held that the molecule does not possess a permanent moment, the results arising because of polarization of the electron clouds around the oxygen atoms in the applied electric field (Paoloni, 1958; Charney, 1961). Hydroquinone possesses a permanent dipole moment (1.4 debyes) but this is less than expected because the partial double bond character of the C—O bonds limits free rotation of the hydroxy groups (Lander and Svirbely, 1945). The dipole moments for the other common quinones are: *o*-benzoquinone 5.1 debyes, 1,2-naphthoquinone 5.6 debyes, and 1,4-naphthoquinone 1.3 debyes. Assuming these values and applying a molecular orbital treatment, Kuboyama (1958, 1959) has calculated the charge distributions over the molecules. The fractional electronic charge on the oxygen atoms was determined as lying between −0.46 and −0.55, and on the carbon atoms of the C=O bond between +0.27 and +0.35, the residual positive charge being distributed throughout the remainder of the ring carbon atoms.

Substitution of a hydroxy or amino group in a position adjacent to the carbonyl group usually provides conditions suitable for hydrogen bonding. Such intramolecular hydrogen bonds have been demonstrated spectroscopically in 2-hydroxy-1,4-naphthoquinone and naphthazarin (Brock-
mann and Franck, 1955), and may possibly be of importance in stabilizing certain structures or altering the reactivity of the quinones. It is interesting to note that lawsone, usually written as 2-hydroxy-1,4-naphthoquinone, is in tautomeric equilibrium with 4-hydroxy-1,2-naphthoquinone:

\[
\begin{align*}
\text{OH} & \quad \text{O} \\
\text{1,4-NQ} & \quad \text{1,2-NQ}
\end{align*}
\]

The 1,4-NQ form is more stable by about 3.8 kcal/mole so that the ratio, \((1,4\text{-NQ})/(1,2\text{-NQ})\), is around 500, and this greater stability may be due in part to the possibility of hydrogen bonding in the 1,4-NQ form. Indeed, Lawsone possesses certain properties indicating that it exists to some extent in the 1,2-NQ form. 2-Amino-1,4-naphthoquinone would presumably also exhibit prototropy.

**Ionization**

The hydroquinones are generally weak acids, as may be seen in the accompanying tabulation taken from the results obtained by Baxendale and Hardy (1953) at 25° and ionic strength 0.65. The \(pK_a\) of p-benzoquinone is undeterminable but was assumed to be greater than 11 by

<table>
<thead>
<tr>
<th>Hydroquinone</th>
<th>(pK_{a_1})</th>
<th>(pK_{a_2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>(p)-Benzohydroquinone</td>
<td>9.85</td>
<td>11.39</td>
</tr>
<tr>
<td>Toluhydroquinone</td>
<td>10.05</td>
<td>11.62</td>
</tr>
<tr>
<td>Durohydroquinone</td>
<td>11.25</td>
<td>12.82</td>
</tr>
<tr>
<td>2,6-Dichloro-(p)-benzo-</td>
<td>7.30</td>
<td>9.99</td>
</tr>
<tr>
<td>hydroquinone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,4-Naphthohydroquinone</td>
<td>9.37</td>
<td>10.93</td>
</tr>
</tbody>
</table>

Michaelis *et al.* (1938). The ionization of catechol \((pK_{a_1} = 9.12\) and \(pK_{a_2} = 12.08\) at 30°) is similar to that of hydroquinone. It is evident that the introduction of electronegative groups increases the acidity, as in the phenol series, and this should be taken into account in considering penetration into cells. The approximate concentrations of the ionized species in a 10 mM solution of \(p\)-benzohydroquinone at pH 7.4 and 37° would be \(p\text{-OH}^- = 0.047\) mM and \(p\text{-Q}^+ = 0.0000063\) mM. However, with the halo and nitro derivatives, for example, the fractions in the ionized forms will be much greater. The cation, \(O\square\text{-OH}^+\), exists only under strongly
acidic conditions \((pK_a = 1.0)\) and is not physiologically important (Biedermann, 1956). The hydroxynaphthoquinones are usually ionized fairly readily; e.g., the \(pK_a\) of lawsone is 3.85, of juglone 8.0 (Friedheim, 1934), and of lapachol 5.02 (Fieser et al., 1948). Thus some of these compounds may exist as anions at physiological pH. The \(pK_a\)'s of various quinones decrease around 0.13 unit with \(10^0\) rise in the temperature (Baxendale and Hardy, 1953).

**Solubility**

The solubilities of the quinones depend on the pH, the salt concentration, and the temperature. \(p\)-Benzoquinone and \(p\)-benzohydroquinone are soluble in water to the extent of around 120 mM and 630 mM, respectively, at 25\(^\circ\) (Ellinger, 1923; Linderstrom-Lang, 1924; Korman and LaMer, 1936). Substitution of \(p\)-benzoquinone with methyl groups progressively reduces the aqueous solubility so that duroquinone is poorly soluble. 1,4-Naphthoquinone is reasonably soluble in water (22 mM) (Ansbacher et al., 1942) but much less soluble in 0.01 \(M\) HCl and 0.09 \(M\) KCl (0.67 mM) (Kläning, 1955). Menadione and menadiol are soluble to the extent of 7.4 mM and 26 mM, respectively (Ansbacher et al., 1942). Menadione bisulfite and menadiol diphosphate are very soluble in water, whereas menadiol diacetate is quite insoluble. Many of the biologically important quinones with several hydrophobic groups or long hydrocarbon sidechains are virtually insoluble in aqueous media.

**Oxidation-Reduction Reactions**

The \(p\)-Q-\(p\)-QH\(_2\) redox couple has been thoroughly studied because of the use of the quinhydrone electrode for pH measurements and is usually represented by the equation:

\[
p\text{-}Q + 2 H^+ + 2e^- \rightleftharpoons p\text{-}QH_2
\]

This equation describes the oxidation-reduction reaction for all of the quinones except those whose hydroquinones ionize appreciably in the pH range used. The standard oxidation-reduction potential of \(p\)-benzoquinone is quite high \((E_0 = + 0.699 \text{ v})\), which means that it is a potent oxidizing agent, and this is fundamentally due to the restoration of aromatic resonance upon reduction. The resonance energy of \(p\)-benzohydroquinone is around 36 kcal/mole, but of \(p\)-benzoquinone only 3–4 kcal/mole; the difference in energy corresponds roughly to the energy involved in the oxidation-reduction reaction. The different redox potentials of the various quinones relate to the resonance energy differences between the quinone and hydroquinone states (Berliner, 1946). The potentials depend, of course, on the pH and in general are 0.40–0.42 v less at pH 7 relative to the standard state \((pH = 0)\) for the benzoquinones; the differences are some-
what greater, perhaps 0.45–0.49 v, for the naphthoquinones. Occasionally insufficient attention has been given to the variations in the redox potential with the pH in metabolic studies; for example, it is possible, for the potential of a quinone within a cell to be 60–100 mv greater than in the external medium. If oxidation of enzyme groups is indeed responsible for some of the effects observed, it is clear that the pH would be an important factor. Oxidation-reduction potentials of quinones commonly used in metabolic studies are summarized in Table 5-1.

The redox potentials of the 1,2- or o-quinones are usually about 90 mv higher than those of the corresponding 1,4- or p-quinones, and thus have been said to have a higher energy content. However, possibly the cause is the hydrogen bonding in the o-hydroquinones, this stabilizing the reduced forms (Badger, 1954). Introduction of groups on the quinone ring alters the redox potentials by inductive effects; electronegative groups reduce the potentials and electropositive groups raise the potentials, the effects being more marked in the naphthoquinones than in the benzoquinones. The effects of substitution on the values of \( E'_0 \) in the physiological range of pH will also depend occasionally on alterations of the \( pK_a \)'s. Recent discussions of the relation of redox potential to structure involve molecular orbital calculations of the lowering of the \( \pi \)-electron energy upon reduction (Basu, 1956), but this is only another way of expressing changes in resonance energy. Particularly valuable reviews of the theories of quinone redox potentials are those of Preisler (1939), Branch and Calvin (1941, p. 305), and Evans and De Heer (1950).

Although the values of \( E_0 \) would indicate most of the quinones to be rather potent oxidants, the potentials at physiological pH's are in an intermediate range, except for o- and p-benzoquinone, being somewhere between the cytochromes \( (E'_0 \) for cytochrome b is + 0.077 and for cytochrome c is + 0.25 v) and the flavins \( (E'_0 \) values around —0.21 v) and pyridine nucleotides \( (E'_0 \) for NAD is —0.32 v), so that certain quinones and naphthoquinones are perhaps ideally poised for participation in electron transport from the flavoproteins to the cytochromes. The potentials for most benzoquinones are certainly higher than for the majority of thiols \( (E'_0 \) values between 0 and —0.35 v; see page II-656), so that the equilibrium

\[
Q + 2 R—SH \rightleftharpoons QH_2 + R—S—S—R
\]

would generally lie far to the right. However, certain other considerations must be taken into account: (1) the rate of oxidation may be slow for various reasons and equilibrium unattained, (2) certain naphthoquinones have reasonably low potentials at pH 7 and would not oxidize some SH groups, and (3) since we know very little about the redox potentials of enzyme SH groups it is difficult to predict the effects on these groups.

Oxidation-reduction reactions of quinones usually involve an interme-
<table>
<thead>
<tr>
<th>Quinone</th>
<th>$E_0$ (pH = 0)</th>
<th>$E_0'$ (pH = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>o-Q</td>
<td>+ 0.792</td>
<td>+ 0.378</td>
</tr>
<tr>
<td>p-Q</td>
<td>+ 0.699</td>
<td>+ 0.284</td>
</tr>
<tr>
<td>Methyl-p-Q (toluquinone)</td>
<td>+ 0.644</td>
<td>+ 0.24</td>
</tr>
<tr>
<td>2,5-Dimethyl-p-Q (p-xyloquinone)</td>
<td>+ 0.589</td>
<td>+ 0.17</td>
</tr>
<tr>
<td>2,3,5-Trimethyl-p-Q (cumnoquinone)</td>
<td>+ 0.526</td>
<td>—</td>
</tr>
<tr>
<td>Tetramethyl-p-Q (duroquinone)</td>
<td>+ 0.452</td>
<td>+ 0.05</td>
</tr>
<tr>
<td>Chloro-p-Q</td>
<td>+ 0.736</td>
<td>—</td>
</tr>
<tr>
<td>2,6-Dichloro-p-Q</td>
<td>+ 0.716</td>
<td>—</td>
</tr>
<tr>
<td>Tetrachloro-p-Q (chloranil)</td>
<td>+ 0.703</td>
<td>+ 0.29</td>
</tr>
<tr>
<td>Hydroxy-p-Q</td>
<td>+ 0.587</td>
<td>—</td>
</tr>
<tr>
<td>2,5-Dihydroxy-p-Q</td>
<td>+ 0.443</td>
<td>+ 0.03</td>
</tr>
<tr>
<td>2,5-Dimethoxy-p-Q</td>
<td>+ 0.465</td>
<td>+ 0.06</td>
</tr>
<tr>
<td>2,6-Dimethoxy-p-Q</td>
<td>+ 0.513</td>
<td>—</td>
</tr>
<tr>
<td>5-Methoxy-TQ</td>
<td>+ 0.542</td>
<td>+ 0.13</td>
</tr>
<tr>
<td>Thymoquinone</td>
<td>+ 0.507</td>
<td>—</td>
</tr>
<tr>
<td>Ubiquinone</td>
<td>+ 0.527</td>
<td>+ 0.113</td>
</tr>
<tr>
<td>Tocopherylquinone</td>
<td>+ 0.77</td>
<td>+ 0.35</td>
</tr>
<tr>
<td>1,2-NQ</td>
<td>+ 0.577</td>
<td>+ 0.127</td>
</tr>
<tr>
<td>1,2-NQ-4-sulfonate</td>
<td>+ 0.637</td>
<td>—</td>
</tr>
<tr>
<td>1,4-NQ</td>
<td>+ 0.483</td>
<td>+ 0.07</td>
</tr>
<tr>
<td>2-Methyl-1,4-NQ (menadione)</td>
<td>+ 0.407</td>
<td>+ 0.02</td>
</tr>
<tr>
<td>2,3-Dimethyl-1,4-NQ</td>
<td>+ 0.339</td>
<td>—</td>
</tr>
<tr>
<td>2-Methoxy-1,4-NQ</td>
<td>+ 0.352</td>
<td>—</td>
</tr>
<tr>
<td>2-Chloro-1,4-NQ</td>
<td>+ 0.508</td>
<td>+ 0.08</td>
</tr>
<tr>
<td>2,3-Dichloro-1,4-NQ</td>
<td>+ 0.499</td>
<td>—</td>
</tr>
<tr>
<td>2-Hydroxy-1,4-NQ (lawsone)</td>
<td>+ 0.356</td>
<td>—</td>
</tr>
<tr>
<td>2,3-Dihydroxy-1,4-NQ</td>
<td>+ 0.288</td>
<td>—</td>
</tr>
<tr>
<td>5-Hydroxy-1,4-NQ (juglone)</td>
<td>+ 0.367</td>
<td>+ 0.033</td>
</tr>
<tr>
<td>2-Methyl-3-hydroxy-1,4-NQ (phthicol)</td>
<td>+ 0.300</td>
<td>—</td>
</tr>
<tr>
<td>2-Amino-1,4-NQ</td>
<td>+ 0.273</td>
<td>—</td>
</tr>
<tr>
<td>1,4-NQ-2-sulfonate</td>
<td>+ 0.552</td>
<td>—</td>
</tr>
<tr>
<td>Lapachol</td>
<td>+ 0.300</td>
<td>—</td>
</tr>
<tr>
<td>Lomatol</td>
<td>+ 0.300</td>
<td>—</td>
</tr>
<tr>
<td>Vitamin K₁</td>
<td>+ 0.328</td>
<td>—</td>
</tr>
<tr>
<td>9,10-AQ</td>
<td>+ 0.140</td>
<td>—</td>
</tr>
<tr>
<td>9,10-PAQ</td>
<td>+ 0.458</td>
<td>+ 0.06</td>
</tr>
</tbody>
</table>

*a These values were all obtained at 25°. The potentials fall with a rise in the temperature and would be roughly 0.009 v less at 37°. The potentials were collected from a variety of sources including Ball (1936), Berliner (1946), Dam and Sondergaard (1960), Friedheim (1934), Harvey (1929), Herz (1954 b), Preisler (1939), and Wallenfels and Möhle (1943).
Diather free radical semiquinone, the stability of which depends mostly on the nature of the quinone and the pH, and may be such that the semiquinone can exist in appreciable concentrations (Michaelis, 1935). Some have believed that the semiquinone is the active form in producing certain effects on enzymes, and in view of the particular reactivities of such free radicals it is necessary to take them into account. Semiquinones may (1) be oxidized to the quinone, (2) reduced to the hydroquinone, (3) dimerize to form quinhydrone, or (4) react with various substances present:

\[
\text{Quinhydrone} \\
\begin{array}{c}
\text{Q} \leftrightarrow \text{QH}^{-} \\
\downarrow \\
\text{Reactions}
\end{array}
\]

\[
\text{QH}^{-} \leftrightarrow \text{QH}_2
\]

and thus the concentration of semiquinone in any system will depend on many factors. The tetrasubstituted semiquinones, such as durosemiquinone, are particularly stable, since the groups sterically hinder dimerization and promote resonance between the semiquinone anions (Wheland, 1955, p. 388). The absorption spectrum of the semiquinone sometimes differs sufficiently from those of the quinone and the hydroquinone so that the semiquinone can be detected photometrically. The semiquinones are, of course, paramagnetic, and this has been used to detect the relatively stable Na\(^+\) salts of several tetrasubstituted semiquinones (Kainer et al., 1956). The hyperfine interaction between the unpaired electron in certain semiquinones with protons has been detected by paramagnetic resonance spectroscopy (Venkataraman and Fraenkel, 1955) and \(p\)-benzosemiquinone finally observed (Blois, 1955). The spectroscopic splitting factor, or \(g\) value, is given for several semiquinones by Blois et al. (1961). The stability of the semiquinones increases with increase in the pH since resonance is favored in the anionic forms. The free energy changes for each of the two steps in the oxidation of a hydroquinone are different. The total 33 kcal/mole involved in the oxidation of \(p\)-benzohydroquinone could be divided by Fieser (1930 b) as follows:

\[
\begin{align*}
\text{QH}_2 & \rightleftharpoons \text{QH}^{-} + \text{H}^{-} & \Delta F &= 22.5 \text{ kcal/mole} \\
\text{QH}^{-} & \rightleftharpoons \text{Q} + \text{H}^{-} & \Delta F &= 10.5 \text{ kcal/mole}
\end{align*}
\]

From the equilibrium constants for the two reactions, it was calculated that the concentration of semiquinone in a solution containing 100 mM of both \(p\)-benzoquinone and \(p\)-benzohydroquinone would be around 0.004 mM.
Some quinhydrone exists in all mixtures of p-benzoquinone and p-benzohydroquinone but under most experimental conditions its concentration is probably less than 10% of the other components and, especially as it appears to be unreactive, is not important in enzyme studies. The structure and properties of quinhydrone are well summarized by Janz and Ives (1961).

The kinetics of quinone oxidation-reduction reactions are usually quite complex and here we shall discuss briefly only a few examples to illustrate that various species of molecule, ion, and free radical are involved. The oxidation of p-benzohydroquinone by Fe+++ and the reverse reaction have been studied in detail. The rate expressions were given by Porret (1934) as:

Reduction of p-Q \quad v = k(Fe^{++})^{3/2} (p-Q) (H^+)^{1/2}
Oxidation of p-QH₂ \quad v = k'(Fe^{+++})^{3/2} (p-QH₂) (H^+)⁻¹

The reaction mechanism was established by Baxendale et al. (1951) to be:

\[ p\text{-QH}^- + Fe^{+++} \rightleftharpoons p\text{-QH}^- + Fe^{++} \]
\[ p\text{-QH}^- \rightleftharpoons p\text{-Q}^- + H^+ \]
\[ p\text{-Q}^- + Fe^{+++} \rightleftharpoons p\text{-Q} + Fe^{++} \]

but other reactions of the semiquinone usually complicate the kinetics. The ratio \( k'/k \) is 430 for p-benzoquinone and 7400 for toluquinone (Baxendale and Hardy, 1954). The rates of oxidation of substances by the quinones are often slow, although the free energy change may be large, due to various kinetic factors and the low concentrations of certain intermediates.

The oxidation of hydroquinones by oxygen can also be kinetically complex, as shown by the extensive work of Weissberger and his colleagues at the Kodak laboratories. With tetrasubstituted hydroquinones, such as durohydroquinone, the products are the respective quinones and hydrogen peroxide:

\[ DQH₂ + O₂ \rightarrow DQ + H₂O₂ \]

but if ring positions are free they may be attacked by the peroxide to form hydroxyquinones and eventually humic acids (James and Weissberger, 1938). The rate of autoxidation increases with the number of methyl groups, this possibly being related to the steady-state concentrations of the semiquinones (James et al., 1938). The reaction is often autocatalyzed by the quinone so that the rate increases after an initial lag period (Weissberger, 1948). Metal ions such as Cu+++ and Mn+++ exert quite marked catalytic effects, while cysteine and thioglycolate are inhibitory, the latter effect probably being due to reaction of the thiols with the autocatalytic quinones formed. In the absence of metal ions, autoxidation of p-benzo-
hydroquinone is slow up to pH 7.8 but becomes very appreciable as the pH is raised above this (LaMer and Rideal, 1924). The rate seems generally to be proportional to (OH⁻)². During such reactions and in most mixtures of quinones and hydroquinones there are several species present, as was well shown in the study of LuValle and Weissberger (1947 a, b, c), and in enzyme or tissue preparations it is frequently difficult to determine which are the active forms, since all these species are formed to varying degrees whatever is added initially unless special precautions are taken.

Stability

Quinones are generally unstable and in solution often polymerize readily during oxidation in air to give brown amorphous products. o-Benzquinone is so highly reactive with water that it is difficult to convert catechol to it in solution, and 1,2-naphthoquinone cannot be recrystallized without undergoing some decomposition, while in acid solutions it dimerizes. The rates of decomposition of p-benzoquinone in various buffers and at different pH’s were determined by Meunier and Queroix (1924). The decomposition is usually accelerated above pH 6.5. Half of the quinone goes to the hydroquinone but the fate of the rest is not surely known. Quinones are more stable in the dark than in the light. Hooker (1936) showed that light induces dehydrogenation at position 3 on 2-hydroxy-1,4-naphthoquinone, this leading to dimerization, while if oxygen is present there is formation of 2,3-dihydroxy-1,4-naphthoquinone.

The antibacterial activity of p-benzoquinone in solution falls with time, about 30% of the activity being gone in 2 weeks and 50% in 7 weeks (Cooper and Haines, 1928). The reactions are complex and involve oxidation-reductions and polymerizations. 1,2-Naphthoquinone in dilute alcoholic buffer solutions decomposes completely in 3 hr into equimolar amounts of 1,2-naphthohydroquinone and 2-hydroxy-1,4-naphthoquinone (Fieser and Peters, 1931). Such reactions must be borne in mind in storing and using quinones for metabolic studies, and indicate that it is usually advisable to make up solutions immediately before use, and to store these for short times, if necessary, in the dark and at low temperatures under nitrogen.

<table>
<thead>
<tr>
<th>Quinone</th>
<th>O₂ uptake over 30 min (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Q</td>
<td>6</td>
</tr>
<tr>
<td>TQ</td>
<td>4</td>
</tr>
<tr>
<td>p-XQ</td>
<td>1</td>
</tr>
<tr>
<td>1,4-NQ</td>
<td>8</td>
</tr>
<tr>
<td>MD</td>
<td>1</td>
</tr>
<tr>
<td>2,3-Dihydroxy-1,4-NQ</td>
<td>65</td>
</tr>
</tbody>
</table>
The spontaneous oxidation of quinones in experimental media leads to an uptake of oxygen. Herz (1954 b), while studying the actions of various quinones on enzymes, found that correction had to be made for the non-enzymic oxygen uptake (see accompanying tabulation). These results are for pH 7.3 and 37°; we have seen that the oxidation rate is strongly dependent on the pH. The theoretical uptake for complete oxidation is 134 µl. Wosilait and Nason (1954) isolated a NADH: quinine oxidoreductase but found that the nonenzymic oxidation of some quinones represented a major fraction of the NADH oxidation (see accompanying tabulation).

<table>
<thead>
<tr>
<th>Quinone</th>
<th>Rate of NADH oxidation (μF₃₄₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nonenzymic</td>
</tr>
<tr>
<td>p-Q</td>
<td>12</td>
</tr>
<tr>
<td>TQ</td>
<td>6</td>
</tr>
<tr>
<td>p-XQ</td>
<td>0</td>
</tr>
<tr>
<td>2,5-Dichloro-p-Q</td>
<td>180</td>
</tr>
<tr>
<td>2,6-Dichloro-p-Q</td>
<td>235</td>
</tr>
<tr>
<td>1,2-NQ</td>
<td>60</td>
</tr>
<tr>
<td>1,4-NQ</td>
<td>0</td>
</tr>
<tr>
<td>MD</td>
<td>0</td>
</tr>
</tbody>
</table>

Inasmuch as the rate of NADH oxidation varies hyperbolically with the quinone concentration for the enzymic reaction and linearly for the non-enzymic reaction (as in Fig. I-2-1), the relative importance of the two reactions will depend on the quinone concentration. These results indicate the need for strict controls in most studies on the quinones, but unfortunately these controls are not included in many reports.

Purification

Commercial samples of the quinones are often not sufficiently pure for accurate work, and some generally useful procedures will be mentioned. Methods for preparation and purification of several benzo- and naphthoquinone can be found in Organic Syntheses. A simple and satisfactory method for purifying p-benzoquinone is sublimation, large crystals forming on a watchglass placed over a beaker of the warmed material; this is preferably done in vacuo. p-Benzoxquinone may also be crystallized from warm water, alcohol, benzene, or ligroin; other quinones can often be purified similarly. Naphthoquinones and their substituted derivatives are sometimes better recrystallized from ether. The water-soluble sulfonate and diphosphates can usually be crystallized as the K⁺ or Na⁺ salts from concentrated warm solutions of KCl or NaCl.
Detection and Determination

Only brief mention of the various ways in which quinones can be determined will be made, since the methods are well described in the standard works on organic analysis. The 1,4-quinones have rather intense absorption bands around 240–245 m\(\mu\), which are shifted to somewhat longer wavelengths by alkylation, and reduction to the hydroquinones brings about marked changes in the absorption, with new peaks appearing around 290–295 m\(\mu\). Ultraviolet absorption measurements may be of use for characterizing the state of oxidation in a system or determining the purity of samples, but are not of much use for quantitative determination of the quinones in complex mixtures. The quinones may be treated in various ways preparatory to spectrophotometric determination. For example, 1,4-naphthoquinone may be determined at 583 m\(\mu\) following reaction with malononitrile, this detecting concentrations as low as 0.003 mM (Gonter and Petty, 1963). Menadione may be analyzed in biological material by reacting with 2,4-dinitrophenylhydrazine with determination of the absorbance at 635 m\(\mu\), this being useful in the range 2–300 \(\mu\)g (Sathe et al., 1957).

Polarographic methods are frequently applicable to quinones. Half-wave potentials for many quinones are given by Kolthoff and Lingane (1952, p. 699), and a chapter is devoted to quinones in the book by Brezina and Zuman (1958, Chapt. XIV, p. 185). Colorimetric methods are well covered in a chapter on the quinones in the book by Snell and Snell (1953). Many spot tests have been devised for the quinones and hydroquinones — e.g., with Liebermann’s reagent, phloroglucinol, or o-phthalaldehyde — and vary greatly in sensitivity and specificity (Feigl, 1960). Most spot tests can detect 1–10 \(\mu\)g of a hydroquinone. Paper chromatography was used by Sproston and Bassett (1954) for the detection and determination of various substituted naphthoquinones, and the \(R_f\) values for these and some benzoquinones are tabulated. In certain systems, hydroquinones may be easily determined by reduction of Fe\(^{+++}\) and reaction of Fe\(^{++}\) with \(o\)-phenanthroline to form the red complex; this method was used by Wosilait and Nason (1954) in their enzyme studies.

REACTIONS OF THE QUINONES

The tanning of skin by quinones and the concurrent physical changes in the proteins stimulated extensive studies many years ago on the reactions of the quinones with proteins, characteristic protein groups, and amino acids. Additional impetus was supplied by the organic chemists who used the quinones and hydroquinones extensively in synthetic procedures. The primary points of attack on the proteins are presumably the SH and the amino groups.
Reactions with Thiols

Quinones do not possess aromatic characteristics and behave chemically more like open-chain $\alpha$, $\beta$-unsaturated ketones, reaction with many substances being by 1,4 addition:

\[
\begin{align*}
1 & \quad 2 & \quad 3 & \quad 4 \\
O=\text{C—C=}& \quad \text{—} \\
\end{align*}
\]

It is thought that direct addition to the ethylenic bond is not common and is biologically unimportant. Quinones may either oxidize thiols or react with them by 1,4 addition, and it is often difficult to distinguish between these reactions in enzyme work. It is often thought that simple oxidation of SH groups should be reversible upon removing the quinone and adding a reductant, whereas 1,4 addition should be irreversible. However, in the case of proteins or enzymes it is not at all necessary that oxidation be readily reversible since secondary structural changes may occur which prevent subsequent reduction. Furthermore, although most addition compounds are reasonably stable, it is not at all certain that the reaction is irreversible. Hydrolysis of the thioquinones could result in the restoration of the thiol, with formation of hydroxyquinones. Indeed, Bray and Garrett (1961) have shown that the addition compound of menadione and glutathione is hydrolyzed by liver glutathionase. Thus one cannot use the criterion of reversibility with assurance in attempting to distinguish between these two types of reaction.

The primary 1,4 addition of a thiol to a quinone may be represented by the reactions:
The forms (I), (II), and (III) are tautomeric isomers and are in equilibrium, but (III) appears to be much more stable than the others, due to the aromatic resonance energy, so that it is the dominant product. The thiohydroquinone (III) will then usually be oxidized to the quinone, either by unreacted quinone:

\[
\begin{align*}
\text{O} & \quad \text{O} \\
\text{S} & \quad \text{R} \\
\end{align*}
\]

especially if it is in excess, or by oxygen (which may be catalyzed by metal ions or enzymes) (Snell and Weissberger, 1939). The degree of oxidation by the unreacted quinone will depend not only on the relative concentrations of quinone and thiol, but also on the relative redox potentials of the quinone and thioquinone. In most cases of biological interest, the thioquinone seems to be the major product, in which case the over-all reaction may be written as:

\[
\begin{align*}
2 & \quad \text{O} \\
& \quad \text{O} \\
\text{S} & \quad \text{R} \\
\end{align*}
\]

Of course, the hydroquinone formed may be reoxidized in various ways, so that the reaction will go entirely to the thioquinone if there is sufficient thiol.

A further reaction which often occurs is the addition of thiol to the thioquinone, followed by oxidation as above, to form a dithioquinone, and this process may continue until all available positions are substituted. Thus Blackhall and Thomson (1953) obtained the following products:

from the reactions of thioglycolate with \(p\)-benzoquinone and 1,4-naphthoquinone. Whether one finds the quinone or the hydroquinone depends on the amount of thiol and its ability to reduce the substituted quinone, as Schubert (1947) demonstrated with the colorless hydroquinone tetrathioglycolate — if this compound is oxidized to the red quinone form, it is
reduced by any excess thioglycolate present. A variety of products can thus be formed from simple 1,4 addition of thiols and the terminal result will depend on many factors. One important fact emerging from the many reports on such reactions is that the rates are often quite high and in many cases the reactions are complete within a matter of several minutes or less.

Substituted groups on the quinone ring exert erratic effects on the rate of thiol addition and the mechanism is often not understood. The occupation of a position usually prevents the addition of the thiol to this position. Thus toluquinone, xyloquinone, cumoquinone, and duroquinone add progressively fewer thiol molecules. Cumoquinone, with one free position, reacts readily with one molecule of thioglycolate, but duroquinone, with no free positions, does not (Snell and Weissberger, 1939). However, in neutral solution, duroquinone oxidizes the SH groups, forming durohydroquinone. In the same way, menadione adds one thiol at the 3-position. If menadione and thioglycolate are mixed, a crystalline thionaphthoquinone:

![Thiohydroquinone](image)

is formed (Fieser, 1941; Fieser and Turner, 1947), and similar compounds are found with other thiols. In contrast to some other addition reactions, the presence of certain groups on \( p \)-benzoquinone or on the 2-position of 1,4-naphthoquinone may actually enhance the 1,4 addition of thiols. Thus menadione reacts with thiols faster than does 1,4-naphthoquinone. On the other hand, a large group, such as a tert-butyl on the 2-position, sterically prevents the addition (Blackhall and Thomson, 1953). It is rather odd that a methoxy group adjacent to an open position is usually able to inhibit thiol addition (Redfearn and Whittaker, 1962). The important factor was considered to be the state of the electron-deficient position; when the adjacent group is strongly electron-attracting or weakly electron-supplying, reactivity increases. Methoxy groups elsewhere do not inhibit; thus 2-methyl-5-methoxy-\( p \)-Q and 2-methyl-5,6-dimethoxy-\( p \)-Q react readily with cysteine. It is interesting and particularly important in considering the mechanism by which certain compounds inhibit enzymes that not all groups shield a position from reaction with thiols. Thus Colwell and McCall (1945) obtained evidence that 2-methyl-3-chloro-1,4-NQ reacts with cysteine and thioglycolate, and Nickerson et al. (1963) have recently shown that 2,3-dichloro-1,4-NQ reacts readily with glutathione, the chlorine atoms being displaced. On the other hand, no reaction with menadione substituted in the 3-position with methyl, methoxy, or hydroxy groups occurs.
Groups elsewhere on the naphthoquinone rings influence the addition; thus juglone reacts with thioglycolate to form the 3-thiojuglone whereas juglone acetate forms the 2-thiojuglone derivative, the acetyl group making the ketone groups asymmetrical (Thomson, 1951).

2-Substituted 1,4-naphthoquinones react readily with glutathione to yield derivatives containing the glutathionyl group bound at the 3-position via the S atom of the glutathione (Nickerson et al., 1963). The rates and yields depend on the electronegativity of the substituted groups, the reaction mechanism being a nucleophilic substitution. The product of the reaction of menadione and glutathione was isolated and named thiodione, the structure of which is perhaps stabilized by hydrogen bonds between glutamyl and glycol carboxylate groups and the quinone O atoms. The redox potential ($E_0$) is some 13 mv higher for thiodione than for menadione. Thiodione is photolabile and visible light causes the appearance of the burgundy-colored 3-mercaptomenadione and some dihydrothiodione (Strauss et al., 1963).

Reaction of quinones with cysteine may be complicated by subsequent intramolecular rearrangements. Kuhn and Beinert (1944) studied the addition of cysteine to $p$-benzoquinone in order to understand better the inhibition of pyruvate decarboxylase they had previously observed. Mixture of the reactants in equimolar concentrations at 20° and pH 5–6 leads to a loss of the color and odor of the quinone, and the nitroprusside reaction becomes negative. Further addition of $p$-benzoquinone gives a brown coloration, and adding up to 2 moles of quinone per mole of cysteine yields a violet precipitate and the simultaneous appearance of 1 mole of hydroquinone. The violet precipitate was thought to be a benzo-$p$-dihydrothiazine derivative resulting from ring closure through the amino group of the cysteine:

\[
\text{COO}^- \quad \text{NH}_3^+ \quad \text{COO}^- \quad \text{H}_2\text{O} + \text{H}^+
\]

since reaction of $p$-benzoquinone with the ethyl ester of cysteine yields a yellow precipitate of comparable structure. Burton and David (1952) state that excess quinone dehydrogenates the newly closed ring to give a true benzo-$p$-thiazine. Thus the simple 1,4 addition may be complicated by the presence of an amino group at the proper distance. One is reminded of the report of Sullivan (1926) that the reaction of 1,2-naphthoquinone-4-
sulfonate with cysteine shows a high degree of specificity and requires an appropriately situated amino group, as well as the work of Neubeck and Smythe (1944) on the same reaction in which a product with an absorption peak around 500-510 m\(\mu\) was obtained, whereas with glutathione reaction occurred but the absorption curve was quite different. However, with 1,2-naphthoquinones, one is less certain of the nature of the reaction, and Kuhn and Hammer (1951) state that 1,2-naphthoquinone does not react with cysteine. Under certain conditions quinones can react with thiols to form quinone-mercaptols, \(O:q: (SR)\), which are easily oxidized to quinone-disulfones, \(O:q: (SO_2R)\), two SH groups having replaced a quinone O atom, but it is doubtful if such products are formed under physiological conditions (Récsei, 1927).

Reactions with Amino Groups

The reaction of quinones with amines and amino acids has been known since the 1880's and it is generally agreed that the mechanism is a 1,4 addition, usually with subsequent oxidation:

\[
\begin{align*}
\text{OH} & \quad \text{OH} \\
\text{OH} & \quad \text{OH}
\end{align*}
\]

\[
\text{OH} + \text{H}_2\text{N}-\text{R} \rightarrow \text{OH} + \text{NH}-\text{R}
\]

exactly as discussed for the thiols in the previous section. A second addition followed by a second oxidation may occur, producing 2 hydroquinone molecules and utilizing 3 quinone molecules (the hydroquinone may, of course, be reoxidized), as in the classic experiments of Fischer and Schrader (1910), who reacted glycine esters with \(p\)-benzoquinone to form

\[
\begin{align*}
\text{C}_2\text{H}_5\text{OOC}-\text{CH}_2-\text{NH} \\
\text{NH}-\text{CH}_2-\text{C}_2\text{OOC}_2\text{H}_4
\end{align*}
\]

which separated as red crystals from the alcoholic solution. Similar reactions occur with alanine esters and with toluquinone instead of \(p\)-benzo-
quinone. Most of such products are red. That the reaction involves the ketone groups of the quinone was suggested by Cooper (1913) when she observed that no reaction occurs with $p$-benzoquinone dioxime. The role of the amino group of the amino acid was confirmed by showing that prior reaction of the amino acid with formaldehyde abolishes the 1,4 addition. The rate of the 1,4 addition of various amines depends on the $pK_a$ of the amino group (Mason, 1955). The relative reactivities of amino acids and related compounds with $p$-benzoquinone and toluquinone were given by Cooper and Haines (1928) (see accompanying tabulation). Indoles with

<table>
<thead>
<tr>
<th>Compound</th>
<th>Relative reactivity ($p$-Q/TQ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylalanine</td>
<td>6.0</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>5.8</td>
</tr>
<tr>
<td>Alanine</td>
<td>5.05</td>
</tr>
<tr>
<td>Glutamate</td>
<td>4.3</td>
</tr>
<tr>
<td>Asparagine</td>
<td>3.95</td>
</tr>
<tr>
<td>Glycine</td>
<td>3.4</td>
</tr>
<tr>
<td>Cystine</td>
<td>2.4</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>2.25</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.6</td>
</tr>
<tr>
<td>Creatine</td>
<td>1.45</td>
</tr>
<tr>
<td>Glycylglycine</td>
<td>1.1</td>
</tr>
</tbody>
</table>

free 3-positions react with various quinones ($p$-Q, o-Q, 1,4-NQ, and 1,2-NQ) to yield intensely colored indolylquinones (Bu’Lock and Harley-Mason, 1951). The reaction sequence was written as:
This type of reaction suggests that the quinones may condense with various cellular components, e.g., certain pyrimidines or purines, but not necessarily through the N atom.

The products of the addition of amino acids to quinones may not be stable and this can result in an over-all degradation of the amino acids. Glycine, for example, is broken down to CO₂, NH₄⁺, and formate by p- and o-benzo-quinones (Blix, 1929). Schönberg et al. (1948, 1949) coined the term "Strecker degradation" (from the discovery by Strecker in 1862 that alloxan degrades alanine) for the formation of aldehydes or ketones containing one less C atom by the action of carbonyl compounds on amino acids. The reaction sequence may be written as (Åkerfeldt, 1953):

\[
\begin{align*}
\text{COO}^- & \quad \text{NH} - \quad \text{C} - \quad \text{R} - \quad \text{COO}^- \\
\text{N} = \quad \text{C} & \quad \text{R} \\
\end{align*}
\]

-2 H

\[+H_2O + H^+\]

\[
\begin{align*}
\text{NH}_2 & \quad + \quad \{ \text{R} \quad \text{CHO} \quad \text{CO}_2 \}
\end{align*}
\]

Åkerfeldt found monoaddition products dominate when most amino acids are reacted with p-benzoquinone, these usually being a deep red and decomposing with the evolution of CO₂, and an aldehyde when excess quinone is present.

1,2-Naphthoquinone-4-sulfonate has been used for the colorimetric determination of amino acids in blood and tissue extracts. It was first prepared by Witt and Kaufmann (1891) and found by them to give colored reaction products with many substances. Several reports appeared between 1900 and 1910 on reactions of this substance with amines and amino acids, and Folin (1922) eventually described a practical analytical test for blood amino acids (as well as emphasizing that the purity of the reagent is essential and giving preparative methods). Sullivan (1926) extended this test and showed that it could be made specific for cysteine, which was the only substance giving a colored product not bleached by hydrosulfite. Obo
(1941 a) wrote the reaction of 1,2-naphthoquinone-4-sulfonate with amino acids as:

\[
\begin{align*}
\text{O} & \quad + \quad H_2N-C\overset{\text{COO}^-}{\text{H}}R \\
\text{H}^+ & \quad \quad + \quad \text{HSO}_3^- \\
\end{align*}
\]

Reaction with cysteine and thioglycolate gives similar products, but with glutathione, glutamate, and glycine the products are spectrophotoscopically much different (Neubeck and Smythe, 1944). o-Benzooquinones apparently react in a comparable manner with amino acids, substitution occurring on the 4- or 5-position, with further complex oxidation and polymerization reactions often occurring (Hackman and Todd, 1953). Proline combines with o-benzoquinone through the ring N to give a quinoneimine derivative (Mason and Peterson, 1955).

The reactions of quinones with amino acids, amines, or other N-containing compounds may thus be quite complex, and there is much more to be learned about the mechanisms involved. Although some of the additions of amino acids are reasonably rapid, in all cases directly compared, the addition of SH groups occurs more readily.

**Reactions with Proteins**

p-Benzooquinone reacts with many proteins (ovalbumin, seralbumin, fibrin, nuclein, legumin, and others) and peptones to give deep red products (Raciborski, 1906). Toluquinone and p-xyloquinone generally behave similarly. Wool proteins, such as keratin, readily react with a variety of quinones to give colored products (Suida, 1913). Gelatin exposed to p-benzoquinone turns yellow and then brown, and loses its ability to dissolve in water, while heat-denatured ovalbumin becomes dark brown and resistant to trypsin and pepsin when so treated (Ellinger, 1923). The mechanism of the antibacterial action of the quinones has been sought in reactions with the bacterial proteins, in a manner somewhat analogous to the tanning of tissues (Cooper, 1913). Reaction of proteins with formaldehyde prevents the formation of colored products with quinones and it was therefore concluded that the amino groups are the sites of attack. The relative reactivity ratio for p-benzoquinone and toluquinone derived from several proteins is around 1.5, less than with most free amino acids (see tabulation on page 441), and much less than when determined with respect to the antibacterial activities (Cooper and Haines, 1928). Hilpert (1925) believed that quinones oxidatively degrade the amino acids of proteins, this causing marked changes in the protein structure and properties. Early work of this type
definitely demonstrated that proteins react with quinones but did not adequately establish the groups involved.

There has been little agreement as to the protein groups reacted by quinones. Despite the fact that SH groups appear to react more readily than amino groups, most emphasis has been placed on the amino groups as the major sites of attack, and actually there is quite a bit of evidence that amino groups are reactive. Several workers before 1930, in addition to those mentioned above, favored the amino group hypothesis, although they presented little concrete evidence. At least half the amino groups on keratin are reacted during exposure to \( p \)-benzoquinone, but if the S—S links are reduced, reaction with the resulting SH groups also occurs (Stoves, 1943). Theis (1945) proposed that \( p \)-benzoquinone reacts with the amino groups of collagen and that this is the important feature of the tanning process, and Green (1953) provided evidence for this by showing that acetylation of the amino groups reduces the binding of quinone. On the other hand, menadione reacts with denatured ovalbumin and this can be prevented by masking the SH groups with iodoacetate or oxidizing them with \( \text{H}_2\text{O}_2 \) (Canady and Roe, 1956). Furthermore, of the 22 amino acids examined, only cysteine reacts readily with menadione, so that this particular quinone apparently is more specific for SH groups. The naphthoquinone can be recovered from the protein complex by alkaline hydrolysis. Certain \( o \)-quinoneimines, such as 2-imino-1,2-fluorenoquinone, seem to react rather specifically with the lysine amino groups of seralbumin, the attachment occurring at the 4-position (Irving and Gutmann, 1953; Gutmann and King, 1962). Some other undetermined amino groups also are reactive. 1,2-Naphthoquinone-4-sulfonate reacts slowly with seralbumin, 14 groups in all being involved, and Belman et al. (1960) believe that 1,4 addition of the lysine amino groups is the major reaction. Mason and Peterson (1955) used \( o \)-benzoquinone (or catechol with tyrosinase) to determine the N-terminal proline residues of proteins. Reaction with such secondary amines occurs readily at neutral pH and the products are characterized by their absorption spectra. The primary amino group, or the peptide bond formed from it, does not react under similar circumstances. Thus only the terminal proline is chromogenic. They demonstrated this reaction particularly with the protein salmine and pointed out a possible relationship to carcinogenesis, since some carcinogens can form quinone-like compounds which might react with nucleoproteins. It is thus perhaps best to conclude with Mason (1955) that both \( N \)- and \( S \)-quinonoid proteins may be formed, the result in any case depending on many factors.

It is interesting to note that Mayer (1950) postulated that quinones are usually quite potent allergenic agents because they combine rapidly with proteins. Olldodart and Rose (1962) have recently prepared an antibody to 1,2-naphthoquinone by coupling it to bovine \( \gamma \)-globulin. This quinone-
globulin antigen elicited antibodies in the rabbit and these were shown to be specific for 1,2-naphthoquinone. The cutaneous anaphylaxis produced by 1,2-naphthoquinone is due to an initial rapid combination with serum proteins. Quinones may alter protein structure by forming interchain or interprotein links:

It is not known how important this is in enzyme work, but certain effects on cells could well be mediated through the formation of such links in the membrane. This subject has been studied with respect to structural proteins, such as collagen and keratin, because of its importance in the tanning industry. Another factor is the autoxidation and polymerization of the quinones, since these polymers are bound to the proteins; this is quite dependent on the pH, increasing from pH 7 to pH 8, below pH 7 only the monomeric quinone being bound (Stecker and Hhighberger, 1942; Theis, 1945). Cross-linking by quinones in keratin fibers was demonstrated by Stoves (1943) by load-extension curves, and several have suggested that cuticular hardening in insects is brought about by e-quinones serving as interfibrillar links. Since most of this work has been done with reasonably high concentrations of quinones (i.e., mostly over 5 mM), it is difficult to predict what would happen at the concentrations generally used in metabolic studies.

**INHIBITION OF ENZYMES**

A variety of enzymes are quite potently inhibited by the quinones but this by no means reflects a single common mechanism of action. Even among the simpler quinones we find different patterns of specificity. Some of the more important results are summarized in Table 5-2, and anyone interested in the relation between structure and action will find many intriguing problems there. The more common mechanisms by which the quinones inhibit enzymes are as follows. (1) *Oxidation of enzyme groups* (see the next section). (2) *Reaction with enzyme SH groups* (see page 463). (3) *Complexing with metal ions.* The formation of complexes between various quinones and the copper of tyrosinase has been demonstrated using Cu$^{64}$ and an ion-exchange resin (Arthur and McLemore, 1956). The e-quinones form more stable complexes than the p-quinones, and this is probably generally true. The inhibition of tryptophan pyrrolase by quinones and
<table>
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<th>Enzyme</th>
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Notes:
- Villela (1963)
- Potter (1942)
- Potter and DuBois (1943)
- Bergstermann and Stein (1944)
- Herz (1954 b)
- Smith and Lester (1961)
- Kashket and Brodie (1963 a)
- Jacobs and Crane (1960)
- Bergstermann and Stein (1944)
- Franke (1944 b)
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Ref. = Reference
polyphenols may be the result of complexes between the substances and the iron of the enzyme (Frieden et al., 1961). In most instances one must take into account complexes formed by the hydroquinones as well as the quinones since both forms will be present. (4) Reaction with substrates or cofactors. The inhibition of the hydrolysis of casein by papain was shown to be due in part to the complexes formed between the casein and hydroquinones, these complexes not being readily attacked by the enzyme (Bahadur and Atreya, 1959). Certain thiol cofactors, such as lipolate or coenzyme A, may be the primary sites of quinone action, although virtually nothing about the reactivity of such cofactors is known. It was suggested that the inhibition of arylamine acetyltransferase may be due to the effects of the quinones on coenzyme A (Guseva and Borikhina, 1958). (5) Production of hydrogen peroxide. The oxidation of hydroquinones by O₂ often forms H₂O₂, which could be the inhibitory agent. This mechanism was postulated for the inactivation of pneumococcal transforming substance (McCarty, 1945) and the inhibition of glycolysis in ascites cells (Pütter, 1963), but it could equally apply to certain enzymes. (6) Nonspecific binding through the aromatic rings. This would presumably be more important for the polycyclic quinones, but might occur to some extent with the benzoquinones, since nonquinoidal aromatic hydrocarbons often interact appreciably with enzymes, as in the interesting study of Miles et al. (1963) on the binding of benzene, naphthalene, and anthracene to chymotrypsin. One would wish to see more controls run with nonquinoidal analogs of the quinones being studied, so that the importance of the quinone groups could be assessed. The inhibition of an enzyme by 1,4-naphthoquinone, for example, cannot be immediately attributed to the quinone structure unless related substances, such as α-naphthol or naphthalene, are shown to be much less active. (7) Competition with quinonoid or polyphenolic substrates (see page 467).

Other mechanisms may be visualized in electron transport systems, but for convenience the effects of quinones on such systems will be deferred to a later section (see page 470).

Oxidation of Enzyme Groups by Quinones

Much attention was given to the redox state of enzyme groups in the early studies, and it was observed that many enzymes, particularly proteinases such as papain, are catalytically active when the SH groups at or near the active center are reduced but inactive when these groups are oxidized (see page II-636). Bersin and Logemann (1933) reported that papain is inactivated by several oxidants, p-benzoquinone abolishing the activity at 1 mM. If a simple oxidation of SH groups were the sole result of treatment of an enzyme with a quinone, activity should be restored by any agent capable of reducing these groups to their original state. Hellerman and
Perkins (1934) found both reversible and irreversible components in the inhibition of papain, sulfide reactivating partially and to an extent determined by various factors. One might expect this to be generally true and the degree of reversibility to depend on the concentration of the quinone, the time of exposure, the pH and many other variables, as well as on the reductant used. It is not known if thiols can directly remove quinones bound to enzymes. An oxidizing mechanism has been thought responsible for the inhibition by p-benzoquinones of intestinal phosphatase (Sizer, 1943 b), cobra venom cholinesterase (Chaudhuri, 1950 b), homogentisate oxidase (Schepartz, 1953), carbonic anhydrase (Chiba et al., 1954 a), and horseradish peroxidase (Klapper and Hackett, 1963), but other workers obtaining comparable results with other enzymes have preferred to assume a 1,4 addition type of mechanism, and indeed the evidence for either mechanism is usually very inadequate or absent. Chaudhuri (1950 b) showed that cholinesterase could be completely reactivated by cysteine, sulfide, or ascorbate, while Klapper and Hackett (1963) found some correlation between the oxidizing potencies of a few quinones and their inhibitory activities against peroxidase. On the other hand, Blagoveshchenskii and Sorokina (1937) could obtain no reversal with cysteine of yeast protease inactivated by p-benzoquinone, and Hochster (1955) could likewise not reactivate xylose isomerase with glutathione. Most of these results are difficult to interpret and, in general, do not prove or disprove any mechanism. Schopfer and Grob (1949 b) considered this problem in some detail with respect to the inhibition of urease, and although they found some correlation between redox potentials and inhibition and showed reversal of 2-chloro-1,4-naphthoquinone-inactivated enzyme, they correctly concluded that one could not adequately distinguish between oxidation and 1,4 addition.

**Reaction of Quinones with Enzyme SH Groups**

Although much has been written of the likelihood of reactions between the quinones and enzyme SH groups, and the quinones have often been classified as SH reagents, it is surprising and disconcerting to find very little experimental evidence for such a mechanism of enzyme inhibition. The concept of the quinones as SH reagents was not seriously considered until the work of Potter and DuBois (1943) on succinate dehydrogenase; it is quite sensitive to a variety of quinones, and the combination with the active site SH group is represented as

![Reaction of Quinones with Enzyme SH Groups](image)
where the + charges are the binding loci for succinate. Substances which are not reactive with SH groups (e.g. 9,10-anthraquinone) or not capable of being oxidized to a quinone structure (e.g. resorcinol) are not inhibitory. The inhibition progresses slowly and is not complete in 60 min when quinone concentrations of 0.01–0.1 mM are used. The presence of succinate delays the inhibition and malonate protects the enzyme against quinones, indicating that the SH group attacked lies in the region binding succinate and malonate. Since malonate does not protect urease against the quinones, direct reaction of the quinones with malonate is disposed of. Quinonoid substances such as p-aminophenol and p-phenylenediamine are equally inhibitory. However, actual proof of such a combination as depicted was not obtained, and other mechanisms can be imagined. The role of oxidation cannot be eliminated since oxidants such as iodine, methylene blue, and ferricyanide are inhibitory, and the oxidation need not involve only the SH groups. Certain amino groups may be particularly reactive on an enzyme surface, and Anderson (1961) has shown that inhibition of alkaline phosphatase by quinones is accompanied by the liberation of ammonia. Some enzymes, e.g. catalase, which are usually not thought of as typical SH enzymes, may be potently inhibited by quinones (Ogura et al., 1950). In the case of catalase no changes in the absorption spectrum occur during inhibition, so the protein was considered to be the site of the reaction. Typical SH enzymes, e.g. 3-phosphoglyceraldehyde dehydrogenase, are quite sensitive to quinonoid substances (Holzer, 1956; Holzer et al., 1956), but the ethylenimino-benzoquinones generally used could react with the enzyme SH groups in a different manner. Furthermore, no direct titration of enzyme SH groups has been carried out with the quinones. One should thus at this time accept the 1,4 addition of enzyme SH groups to quinones with caution, although there is certainly no reason, in view of the rapid reaction of quinones with many thiols, to believe that this may not be an important mechanism.

Reactive Form of the Quinones

A good deal of attention has been paid to determining if the quinone, hydroquinone, or semiquinone is the inhibitory species in particular cases, and this is quite important because of the bearing on the possible mechanisms of the inhibitions. We have pointed out that it is virtually impossible to assure the presence of only one species in most cases, even though very pure quinone or hydroquinone is added to the enzyme preparation, and even though oxygen is carefully excluded in the latter case, since oxidation-reduction reactions with the enzyme or other components of the mixture may occur. However, it is usually possible to obtain reasonably good evidence for the importance of one or the other species. Fischgold (1934) found that p-benzohydroquinone, treated with hydrogen and palladized asbestos to reduce any quinone present, produced no inhibition
of urease even at 18.4 mM, the conclusion being that only the quinone form must be active. Another related approach is to use both the quinone and hydroquinone forms in as pure a state as possible and observe the kinetics of the inhibition. Quastel (1933 b) found, for example, that $p$-benzoquinine is a more potent inhibitor of urease than $p$-benzohydroquinone — 0.0037 mM of the former inhibited 98% and of the latter 64%. If one compares there results with those of Fischgold, it is clear that Quastel’s hydroquin-one sample must have contained quite a bit of quinone; nevertheless, a differential effect like this is indicative if not absolute proof. A somewhat better method is to determine the rates of inhibition, as did Barron (1936) with pyruvate oxidase and Schoetensack (1948) with renal phosphatase, both enzymes being inhibited rapidly by $p$-benzoquinone but slowly by $p$-benzohydroquinone, the delayed action in the latter case presumably being due to the necessity for oxidation of the hydroquinone. Since hydro-quinone oxidation rate decreases with a lowering of the pH, one can sometimes compare inhibitions more profitably at a reduced pH. Thus Potter (1942) showed that at pH 5 there is a marked differential effect of $p$-benzo-quinone and $p$-benzohydroquinone on succinate dehydrogenase — 0.0002 mM of the former inhibits 57% after 20 min while the latter does not inhibit at all. Quastel’s experiments were run at pH 7.4 which may account for his results. In most instances the quinone form has been shown to be the more inhibitory, but Franke (1944 b) reported that $p$-benzoquinone and $p$-benzohydroquinone inhibit some enzymes equally (oxalate oxidase and lipoxidase), the former to inhibit some more potently (D-amino acid dehydrogenase and succinate dehydrogenase), and the latter to inhibit other enzymes more strongly (catalase and yeast lactate dehydrogenase), although it is not possible to draw definite conclusions since the conditions were not ideal. Some have favored reduction of the enzyme as being responsible for inhibition (Takeuchi, 1933; Itoh et al., 1939), but it is more likely that inhibitions by hydroquinones are related to other mechanisms.

The semiquinone form may arise during oxidation-reduction reactions and should at least be considered as the inhibitory species in some cases. It is true that the semiquinones are not as reactive as most free radicals, but they might react with enzymes in ways other than the quinone or hydro-quinone, as Michaelis (1935) emphasized. The participation of the semi-quinone in inhibition will depend not only on the inherent reactivity but as much on the concentration of semiquinone reached in the system, which is dependent on many factors, particularly the pH and the structure of the quinone. This has been studied more carefully in the $p$-phenylenediamines (Kensler et al., 1942 a) and the inhibition of pyruvate decarboxylase was shown to be correlated with the stability of the semiquinone (see page 590). I know of no direct proof for the importance of the semiquinone in enzyme inhibition. Duroquinone, whose semiquinone is the most stable
of the simple semiquinones, has seldom been used in enzyme inhibition studies unfortunately, but when it has, as in the work of Kuhn and Beinert (1947) on pyruvate decarboxylase, it has not proved to be particularly inhibitory.

**Effects of Substituent Groups on Inhibitory Activity**

The changes in activity resulting from the introduction of various groups into benzo- and naphthoquinones (Table 5-2) are difficult to interpret and no general theory has emerged despite the rather voluminous data. The interaction of the quinone with the enzyme may be modified by introduced groups in a number of ways, since these groups alter many properties of the quinones (see page I-304). Some of the more obvious and important properties changed by these groups are: (1) oxidation-reduction potential, (2) ionization of the hydroquinone, (3) semiquinone stability, and (4) reactivity with SH or amino groups. In addition, these groups may exert steric interference with the approach of the quinone to the enzyme surface or, on the other hand, contribute to the binding energy. We shall confine ourselves to a few comments on structure-action relations inasmuch as failure to understand the mechanism of inhibition in most cases prevents a more complete analysis.

The introduction of successive methyl groups, or related groups, into p-benzoquinone almost invariably reduces the inhibitory potency, as seen by examining the data in Table 5-2 for several enzymes, mostly studied by Hoffmann-Ostenhof. It is not known if this is related to the decrease in $E'_0$ in such a series (Table 5-1), a decreased opportunity for the addition of enzyme SH groups, steric interference by the bulky groups, or some other factor. Chlorine groups, in contrast to methyl groups, generally increase the inhibitory activity, and even chloranil is more potent than $p$-benzoquinone. Since chlorine groups do not effect $E'_0$ markedly, the different reactivity must be due to some other factor. Although one might expect chloranil to be unable to add SH groups, one must remember that chlorine atoms are easily displaced (see page 438). There appears to be no clear rule concerning the relative activity of benzo- and naphthoquinones, some enzymes being more sensitive to one and some to the other. However, in ten enzymes for which adequate comparison can be made, 1,2-naphthoquinone is always more inhibitory than 1,4-naphthoquinone, which may or may not be related to the difference in $E'_0$. Masking the keto or hydroxy groups of naphthoquinones usually abolishes inhibition; an example is the failure of menadiol diphosphate to inhibit aldehyde oxidase (Rajagopalan et al., 1962).

If inhibition by 1,4-naphthoquinones is related to a reaction with the enzyme SH groups, one would predict that the introduction of most groups into the 2- and 3-positions to produce 2,3-disubstituted naphthoquinones
would abolish the inhibition. This is borne out in that dihydroxy-1,4-naphthoquinone and 3-hydroxymenadione have relatively little inhibitory activity against succinate dehydrogenase (Herz, 1954 b), and the latter compound has little effect on pyruvate decarboxylase (Kuhn and Beinert, 1947). On the other hand, 2,3-dichloro-1,4-naphthoquinone is usually even more inhibitory than the parent naphthoquinone, which may be correlated with the fact that chlorination actually increases 1,4 addition of SH groups. The effects of various groups substituted in the 2-position of 1,4-naphthoquinone on the inhibition of pyruvate decarboxylase are very marked (Foote et al., 1949) and cannot be correlated entirely with change in the values of $E_0'$. That 2-mercapto-1,4-naphthoquinone is some 145 times more inhibitory than 2-hydroxy-1,4-naphthoquinone is interesting. The low inhibitory activity of lawsone cannot be attributed to its tautomerism with the 1,2-naphthoquinone structure since 1,2-naphthoquinone is as inhibitory as 1,4-naphthoquinone. The introduction of a methyl group into the 2-position to give menadione usually reduces the activity somewhat, as is seen particularly well with urease (Schopfer and Grob, 1949 b), but a chlorine group increases the inhibitory potency about 5 times, these changes possibly being due to the inductive effects of these two groups with respect to making the 3-position reactive with SH groups.

**Formal Nature and Kinetics of Inhibitions**

In a surprising number of instances the inhibition by quinones has been found to be formally competitive; examples are carbonic anhydrase (Chiba et al., 1954 b) and the phosphate-activated renal glutaminase (Sayre and Roberts, 1958). In the latter case, p-benzoquinone apparently competes with the phosphate and not the substrate. Some enzymes are competitively inhibited by hydroquinones, for example catechol oxidase (Kuttner and Wagreich, 1953) and D-amino acid oxidase (Frisell et al., 1956). A competitive relationship for catechol oxidase is of course not unexpected, and the inhibitions produced with certain enzymes acting on phenolic substances or requiring such substances for activity, such as $\beta$-glucosidase (acting on arbutin, salicin, and related glucosides), NADH:H$_2$O$_2$ oxidoreductase, and homogentisate oxidase, are probably competitive. 1,2-Naphthoquinone-4-sulfonate competitively inhibits both dopa decarboxylase (Hartman et al., 1955) and L-glutamate decarboxylase (Roberts and Simonsen, 1963), but this may be more related to the sulfonate group. The inhibition of succinate dehydrogenase must be dynamically competitive, since succinate protects the enzyme, but would be noncompetitive under equilibrium conditions (Potter and DuBois, 1943). Indeed, Redfearn and Whittaker (1962) reported noncompetitive inhibition by two $p$-benzoquinones of a heart succinate dehydrogenase preparation using methylene blue as an acceptor and ubiquinone analogs as mediators.
Noncompetitive kinetics have also been found for the inhibitions of tryptophan pyrrolase (Frieden et al., 1961) and NADH:H₂O₂ oxidoreductase (Gamborg et al., 1961) by p-benzoquinone. Foote et al. (1949) claim that the inhibitions of pyruvate decarboxylase by various 1,4-naphthoquinones depend on the presence of substrate and hence that the inhibitors must combine with the ES complex, the inhibition thus presumably being uncompetitive, but possibly pyruvate augments the inhibition by altering the state of the SH groups involved in the catalysis.

The potent inhibition of succinate dehydrogenase by p-benzoquinone suggests that some structural factor may be involved. p-Benzooquinone is structurally related to succinate or fumarate in that two negatively charged groups are separated by about the same distance in each. Although the O atoms in the quinone are not ionic, they possess an appreciable negative charge (see page 426), and are approximately 5.4 Å apart, which might allow some interaction with the two cationic groups on the enzyme surface, this orienting the quinone so that reaction with the SH group might more readily occur. It would be interesting to know how the potencies of o-benzoquinone and 1,2-naphthoquinone compare with the corresponding 1,4-quinones, and to know whether duroquinone and chloranil inhibit or not, but I have found no data on these points.

The rate of inhibition is fairly slow in most instances, as we have previously noted, and a typical curve for the development of inhibition is that for p-benzoquinone acting on heart lactate dehydrogenase (Fig. 5-1)

![Fig. 5-1. Inhibition of pig heart lactate dehydrogenase by p-Q at 1 mM. The inhibition becomes complete at near 180 min. (From Pileiderer et al., 1959.)](image-url)
INHIBITION OF ENZYMES

(Pfleiderer et al., 1959). Although 50% inhibition is reached in around 10 min, full inhibition does not occur until nearly 180 min. During the progress of the inhibition there is a progressive loss of free SH groups as determined by titration with p-MB. The completely inhibited enzyme is found to have had 14 SH groups reacted, but if the inactivated enzyme is allowed to stand at pH 10, 3 SH groups are recovered, so that 3 SH groups appear to react differently from the others with the quinone. The basic mechanism is not a simple oxidation of SH groups to S—S groups. It is interesting that the treated enzyme shows an altered absorption spectrum; this might be used to determine the extent of reaction of quinones with this enzyme and perhaps others. Inhibition rate curves are shown in Fig. 5-2 for pancreatic α-amylase (Owens, 1953 a). The rate of inhibition by 2,3-dichloro-1,4-naphthoquinone is seen to be almost double that by 1,4-naphthoquinone, but is still quite slow.

![Fig. 5-2. Rates of inhibition of pancreatic amylase by naphthoquinones at 1 mM. (From Owens, 1953 a.)](image)

Very little work on the effects of pH on enzyme inhibition by the quinones has been reported. Curves for catalase and urease are given in Fig. 5-3 (Hoffmann-Ostenhof and Biach, 1948 a) and are so different in configuration that it was postulated the mechanisms of inhibition cannot be the same. Alteration of the pH would not only change $E_0'$ but might modify the rate of reaction with SH groups. The increase in the inhibition of urease from pH 5 to 7 in the face of a decreasing $E_0'$ indicates that factors other than the redox potential are involved, although the curve for catalase might support this mechanism in part. The redox po-
tential of the enzyme SH groups would, of course, also be modified by the pH. However, it seems likely that the state of ionization of the SH groups or the changing over-all charge on the enzyme may be of greater importance. Finally we shall inquire whether mutual depletion kinetics should ever be used in the analysis of quinone inhibition. This will depend on the potency of the inhibition, but obviously in some cases the potency is sufficient for depletion of free inhibitor to occur, and indeed Hoffmann-Ostenhof and Gierer (1948) noted the inhibition of catalase by \( p \)-benzoquinone

\[
\text{Fig. 5-3. Inhibitions of catalase and urease by } p-Q \text{ at different pH's. (From Hoffman-Ostenhof and Biach, 1948 a.)}
\]

at 0.1 mM to be 40% when 3.5 units of catalase were present and only 15% when 11 units of catalase were used. Ackermann and Potter (1949) also found typical pseudoirreversible inhibition of succinate dehydrogenase by \( p \)-benzoquinone, curves such those as in Fig. I-3-14 being obtained. The potent antimalarial naphthoquinones, such as SN-5949, are classic examples of this type of inhibition; they will be discussed elsewhere.

**EFFECTS ON ELECTRON TRANSPORT SYSTEMS**

The disturbances produced by quinones in various electron transport systems are frequently complex because of the different mechanisms which may be involved. The recent demonstration of the functioning of benzoand naphthoquinones in complete electron transport chains has increased the understanding of how certain unnatural quinones can interfere, and therefore a brief summary of the role of the natural quinones will be presented.
Participation of Quinones in Electron Transport Systems

It has been known for many years that certain hydroquinones can donate electrons to specific sites in the electron transport chain, and that quinones can often act as electron acceptors, either being directly reoxidized or passing electrons on to enzymes within or outside the normal phosphorylating sequences. Most of the early work was with \( p \)-benzoquinone and its hydroquinone. The enzyme thought to be concerned with the oxidation of \( p \)-benzohydroquinone was called indophenol oxidase but Stotz et al. (1938) showed the oxidation to be mediated by cytochrome c or other components of the cytochrome portion of the complex. Many enzyme systems isolated from a variety of sources have been shown to interact with quinones; for example, (1) succinate:quinone reductase, (2) NAD(P)H:quinone oxidoreductase (quinone reductase), (3) NAD(P)H:2-methyl-1,4-naphthoquinone oxidoreductase (menadione reductase), (4) \( \text{QH}_2 \):cytochrome c reductase, (5) \( \text{QH}_2 \):cytochrome \( c_1 \) reductase, (6) ferrous iron:quinone reductase, (7) DT diaphroase, (8) vitamin \( K_1 \) reductase, and (9) ubiquinone reductase. Most of these appear to be fragments of normally functioning complete electron transport systems. The quinones occurring in intact electron transport systems are usually benzoquinones of the coenzyme Q (ubiquinone) series or naphthoquinones of the vitamin \( K_1 \) series, although other types of quinone, such as the plastoquinones, the vitamin \( K_2 \) series, and the tocopherylquinones may also participate in some instances.

\[
\text{CH}_3\text{O} \quad \text{O} \quad \text{CH}_2\text{C} = \text{C} \quad \text{CH}_2\text{H} \quad \text{H} \\
\text{CH}_3\text{O} \quad \text{O} \quad \text{CH}_3
\]

**Coenzyme Q (ubiquinone) series**

\[
\text{CH}_3\quad \text{CH} = \text{C} \quad \text{CH}_2\text{H} \quad \text{H} \\
\text{CH}_3\quad \text{CH}_2\text{C} = \text{C} \quad \text{CH}_2\text{H} \quad \text{H} \\
\]

**Vitamin \( K_1 \) series**

The lipid-soluble ubiquinones probably exist in the lipoidal phase of the particulate electron transport systems and transfer electrons between the flavoproteins and cytochrome \( b_1 \). Extraction of mitochondria with
lipid solvents abolishes succinate oxidase and succinate:cytochrome c reductase activities, and restoration of activity can be achieved by adding the extract or functional ubiquinones, but not with the simpler benzoquinones or naphthoquinones (F. L. Crane, 1960; Lester and Fleischer, 1961). The exact role of the naphthoquinones is not yet clear but they appear to function in a comparable region of the electron transport chain, although it is not certain whether they are generally on the main sequence or on bypass chains. In bacteria it has been postulated that the two groups of quinones function as the terminal components of the converging arms of the electron transport system:

\[ \text{NADH} \rightarrow fp_1 \rightarrow \text{vit} K_2 \rightarrow \text{cyto-b}_1 \rightarrow \text{cyto-a}_2 \rightarrow O_2 \]

\[ \text{Succinate} \rightarrow fp_2 \rightarrow UQ_n \]

(Kashket and Brodie, 1963 b), but the situation is different in plant and animal tissues. There are only small amounts of quinones in tissues — e. g. the ubiquinones are usually in the range 1–10 mg/100 g wet weight (Thomson, 1962) — but the concentrations within the electron transport systems may be high.

It is necessary to emphasize the remarkable specificity exhibited by the quinones in these systems. We have noted the relative abilities of various quinones to accept electrons from NADH in a reductase preparation from peas (page 434) (Wosilait and Nason, 1954). A bacterial Fe$^{++}$- and FAD-activated nitrate reductase operates maximally with menadione, or the 2,3- and 2,6-dimethyl-1,4-naphthoquinones, but much less rapidly with lapachol or phthiocol, and not at all with vitamin K$_1$ (Wainwright, 1955). Ernster (1961) found that menadione is the best mediator of electrons in the oxidation of glutamate by rat liver mitochondria, but that various benzo- and naphthoquinones can function to varying degrees and in different pathways; e. g., \( p \)-benzoquinone will function in electron transfer but the system is no longer amobarbital-sensitive. The oxidation of NADPH by a submitochondrial fraction from liver, designated a DT diaphorase, is rapid with menadione, but vitamins K$_1$ and K$_2$, lawsone, 1,2- and 1,4-naphthoquinones, and various benzoquinones including the ubiquinones, are either very slightly active or inactive (Conover et al., 1963 a). These few examples will suffice to illustrate the specificity and to indicate that the interactions between the quinones and the various components of the electron transport chains are controlled by factors such as lipid solubility or enzyme catalysis and not entirely by the relative redox potentials.

The roles played by the quinones in electron transport are discussed in detail in the Ciba Foundation Symposium “Quinones in Electron Transport” (1960), “The Biochemistry of Quinones” edited by Morton (1964), and the reviews by Dam and Sondergaard (1960) and Thomson (1962).
Ways in Which Quinones May Modify Electron Transport

Quinones may either stimulate or depress the rate of electron flow in the total sequence, or differentially alter the rates in different segments of the sequence, even occasionally increasing the flow rate in one region and decreasing it in another. There is probably no group of so-called inhibitors so apt to stimulate metabolism as the quinones.

(A) Directly inhibit enzymes of the sequence. The inhibition of some dehydrogenases has been discussed and other examples may be found in Table 5-2, and quite possibly more distal enzymes are occasionally attacked, even the cytochromes not being immune. The mechanism of the inhibition might be reaction with SH groups, but other mechanisms must also be considered (see page 445).

(B) Compete with or displace natural or endogenous quinones. Such an action would not necessarily lead to inhibition, inasmuch as the exogenous quinone may function in electron transfer even more effectively than the endogenous quinones. The best way to demonstrate such a mechanism is to deplete the system of its natural quinones, and then add back both the natural and the test quinones at different concentrations, as in any experiment designed to establish whether an effect is competitive or not.

(C) Serve as simple electron donors or acceptors. Hydroquinones can feed electrons into an electron transport sequence, while quinones can drain them out of the normal channel of flow:

\[ \begin{align*}
Q & \rightarrow O_2 \\
SH_2 & \rightarrow X_1 \rightarrow X_2 \rightarrow X_3 \rightarrow X_4 \rightarrow O_2 \\
QH_2 & \\
\end{align*} \]

The hydroquinones formed in the latter case might pile up or be directly oxidized by oxygen. The rates of electron flow in different segments of the chain may thus be influenced quite differently. If one is measuring total oxygen uptake, the final result will depend on a variety of factors. If one is measuring the rate of reduction of some acceptor, such as a dye, quinones might exert an apparent inhibition by diverting the flow away from the dye:

\[ \begin{align*}
SH_2 & \rightarrow X_1 \\
\text{dye} & \\
Q & \\
\end{align*} \]

Indeed, the quinones may accept electrons directly from a substrate, as we have noted for NAD(P)H, and it is often important to correct for non-
enzymic substrate oxidation (Kikuchi and Barron, 1959; Koide, 1962). Hydroquinones, either added or formed from quinones, may donate electrons into the sequence, most probably at the cytochrome c level. Williams (1963) has reported a very interesting kinetic study of this reaction, which proceeds in two steps:

\[
\begin{align*}
QH_2 + \text{cyto-}c_{ox} & \rightleftharpoons QH^\cdot + \text{cyto-c}_{red} + H^+ \\
QH^\cdot + \text{cyto-}c_{ox} & \rightleftharpoons Q + \text{cyto-c}_{red} + H^+
\end{align*}
\]

the second proceeding more rapidly than the first. The accelerating effect of quinone on the reaction is attributed to the formation of the semiquinone, \(Q + QH_2 \rightleftharpoons QH^\cdot\). Ionic strength effects and entropy changes indicate that an electron transfer between oppositely charged ions occurs, and it was suggested that the formation of the active complex might involve interactions of the aromatic ring of the hydroquinone with hydrophobic regions of the cytochrome c. T. Nakamura (1960a, b, 1961) has also obtained evidence for the initial formation of the semiquinone in the oxidation of \(p\)-benzohydroquinone by a laccase preparation, and for the importance of the semiquinone in determining the over-all rate of oxidation.

(D) Establish alternate or bypass pathways for electron flow. This is, of course, closely related to the previous mechanism, but specifically it is the introduction of an alternate pathway:

\[
\begin{align*}
&Q \\
\text{SH}_2 & \rightarrow X_1 \rightarrow X_2 \rightarrow X_3 \rightarrow X_4 \rightarrow O_2
\end{align*}
\]

which may have properties quite different from those of the original pathway. A simple example is the acceleration of the reduction of cytochrome c with NADH as a substrate by menadione in an enzyme from lymphosarcoma, and to a lesser extent by \(p\)-benzoquinone and 1,4-naphthoquinone (Strength, 1956). On the other hand, 1,2-naphthoquinone augments the oxidation of NADH but does not reduce cytochrome c. The functioning of \(p\)-benzoquinone as a mediator between NADH and cytochrome c has also been noted in electron transport systems from Fusarium (Kikuchi and Barron, 1959). More complex situations of this type have been observed and are particularly important in oxidative phosphorylations, to be discussed later (page 477).

(E) Block specific site in electron transport sequence. Certain antimalarial 3-alkyl-1,4-naphthoquinones, such as SN-5949, are known to be potent inhibitors of electron transport through a region also sensitive to antimycin A. Although this type of inhibition will be discussed elsewhere, it is well to remember that some of the simpler naphthoquinones also might act to
some extent in this way. The fundamental mechanism may fall into one of the above categories but is at present unknown.

Examples of the Inhibition of Electron Transport

An apparent paradox exists with respect to bacterial NAD(P)H:nitrate oxidoreductase. Wainwright (1955) found a nitrate reductase from *E. coli* to require menadione, or a related naphthoquinone, for activity, but Sadana and McElroy (1957) reported a similar enzyme from *Achromobacter fischeri* to be completely inhibited by 0.03 mM menadione. The discrepancy may be due simply to the fact that the electron transport systems may be quite different in the two organisms, but may also be due to the different electron donors used. Wainwright used methylene blue while Sadana and McElroy used NADH. When the latter workers used reduced benzylviologen as a donor, no inhibition by menadione was observed. Thus it may be that methylene blue feeds into a menadione-requiring region of the system, while a region readily blocked by menadione may exist between NADH and the site at which the dyes donate electrons. This at least illustrates the importance of considering the electron donors and acceptors used, and serves as a warning against carrying over results obtained on artificial systems to intact systems as they occur in the cell.

Certain analogs of coenzyme Q inhibit quite potently the oxidation of succinate in liver mitochondria. \( Q_0, 6-\text{Cl}-Q_0, \) and \( 6-\text{Br}-Q_0 \)* inhibit around 75\% at concentrations near 0.12 mM, while \( Q_1, Q_2, \) and \( Q_3 \) slightly stimulate the rate of oxidation (Jacobs and Crane, 1960). In acetone-extracted mitochondria, which have lost their succinate:cytochrome \( c \) reductase activity, coenzyme Q analogs inhibit the restoration of activity induced by adding the natural quinones (F. L. Crane, 1960). Thus diethoxy-\( Q_{10} \) is inhibitory and this inhibition is antagonized by \( Q_{10} \), but other inhibitions, such as that produced by lapachol, cannot be reversed by \( Q_{10} \), so that the latter inhibitions must be at a different site in the sequence. The reduction of cytochrome \( c \) by succinate is inhibited 82\% by 0.02 mM 6-\text{Br}-\text{Q}_0 and this is prevented by cysteine or glutathione (Smith and Lester, 1961). Both these workers and Jacobs and Crane (1960) feel that reactions of these quinones with SH groups may be important in their actions. The inhibition of succinate oxidation by lapachol and hydrolapachol is released by 2,4-dinitrophenol, and this was thought to be due to a dissociation of the inhibitor complexes (Howland, 1963 b), but possibly the uncoupler shifts the electron flow to a pathway insensitive to these naphthoquinones.

Horseradish peroxidase catalyzes the oxidation of menadiol, reduced vitamin \( K_1 \), and \( Q_{10}H_2 \), and this is inhibited by various quinones (see

\* \( Q_n \) represents coenzyme Q (ubiquinone) with \( n \) indicating the number of isoprenoid units (see formula on page 471).
accompanying tabulation) (Klapper and Hackett, 1963). This is certainly one of the most potent inhibitions by \textit{p}-benzoquinone yet reported.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (mM)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{p}-Q</td>
<td>0.0012</td>
<td>95</td>
</tr>
<tr>
<td>\textit{Q}_6</td>
<td>0.043</td>
<td>95</td>
</tr>
<tr>
<td>MD</td>
<td>0.77</td>
<td>95</td>
</tr>
<tr>
<td>3-\text{CH}_3\text{O}-MD</td>
<td>0.42</td>
<td>36</td>
</tr>
</tbody>
</table>

The inhibition was claimed to be correlated with redox potential but too few inhibitory quinones were used to establish this. There is a great need for more thorough analysis of such systems and the ways in which the quinones can interfere with electron transport. The observation that Mn\textsuperscript{++} reverses the peroxidase inhibitions, for example, cannot be readily interpreted but could be an important clue in the elucidation of the inhibitory mechanism.

\section*{Interactions of Quinones with Metalloflavoproteins}

Quinones can serve as electron acceptors from certain flavoproteins (Wieland and Mitchell, 1930; Booth, 1935), and recent work has demonstrated a variety of quinone reductases. A very thorough investigation of the interactions between quinones and metalloflavoproteins was undertaken by Mahler \textit{et al.} 1955 \textit{a)} and, although they did not study inhibitions in such systems, the results are of obvious importance for inhibition of electron transport. It was found that \textit{p}-benzoquinone, \textit{p}-xyloquinone, 1,4-naphthoquinone, and various substituted 1,4-naphthoquinones can serve as efficient acceptors for several such flavoenzymes. For example, excess NADH in the presence of reduced NADH:cytochrome reductase is oxidized very slowly by oxygen, but the addition of menadione induces a rapid oxidation of the NADH by the following sequence of reactions:

\[
\text{NADH} + \text{fp} + \text{H}^+ \rightleftharpoons \text{NAD}^+ + \text{fpH}_2
\]

\[
\text{fpH}_2 + \text{MD} \rightleftharpoons \text{fp} + \text{MDH}_2
\]

\[
\text{MDH}_2 + \text{O}_2 \rightleftharpoons \text{MD} + \text{H}_2\text{O}_2
\]

The quinones take the place of cytochrome \textit{c} as acceptors from the flavoenzymes but, since they are 2-electron acceptors, no metal ion is involved. The rates are in general quite comparable to those when cytochrome \textit{c} is the acceptor. The reduced quinones can react with cytochrome \textit{c} if it is present. In the total system there would be competition between the quinones and cytochrome \textit{c} as acceptors from the flavoenzymes, as well as
competition between cytochrome c and oxygen for the hydroquinones. The nature of the enzyme, the quinone used, the relative concentrations of flavoenzyme, cytochrome c and quinone, and the conditions of the experiment will determine the patterns of electron flow. The results are summarized in Table 5-3, from which it may be observed that there may be

### Table 5-3

<table>
<thead>
<tr>
<th>Quinone</th>
<th>$E_a'$ (pH 7)</th>
<th>Relative rates of FADH$_2$ oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
</tr>
<tr>
<td>MD</td>
<td>− 0.030</td>
<td>65</td>
</tr>
<tr>
<td>2-Et-1,4-NQ</td>
<td>− 0.020</td>
<td>78</td>
</tr>
<tr>
<td>1,4-NQ</td>
<td>+ 0.036</td>
<td>94</td>
</tr>
<tr>
<td>2-Cl-1,4-NQ</td>
<td>+ 0.056</td>
<td>90</td>
</tr>
<tr>
<td>5,8-diOH-MD</td>
<td>+ 0.075</td>
<td>100</td>
</tr>
<tr>
<td>p-XQ</td>
<td>+ 0.166</td>
<td>61</td>
</tr>
<tr>
<td>3-Me-1,2-NQ</td>
<td>+ 0.075</td>
<td>70</td>
</tr>
</tbody>
</table>

$^a$ All the relative rates are normalized to 100 for the maximal rate. The values for $E_a'$ are as given by Mahler et al. (1955a) and are lower than those in Table 5-1, but the relative values are comparable. The enzymes used are: I = NADH:cytochrome reductase, II = diaphorase, III = L-amino acid oxidase, IV = butyryl-CoA dehydrogenase, V = Mo-free xanthine oxidase, and VI = xanthine oxidase with Mo added. All the quinones were at 0.5 mM.

an optimal $E_a'$ for the acceptor, matching in some manner the flavoenzyme donor, the $E_a'$ for which varies somewhat from enzyme to enzyme. Although the metal ion may not be directly involved in the quinone reaction, it will also be of some importance in determining the over-all eventual electron flow.

### EFFECTS ON OXIDATIVE PHOSPHORYLATION

Various quinones appear to be involved not only in electron transport but in certain phosphorylations, and this provides opportunity for exogenous quinones to interfere with the generation of ATP directly by an uncoupling action.

#### Participation of Quinones in Oxidative Phosphorylation

Mitochondria from the livers of vitamin K-deficient chicks exhibit a reduced P:O ratio ($\beta$-hydroxybutyrate and succinate as substrates) com-
pared to mitochondria from normal livers (Martius and Nitz-Litzow, 1954 a), and the addition of vitamin K₁ to the mitochondria restores the P:O ratio to near-normal levels (Martius and Nitz-Litzow, 1954 b), leading to the postulate that vitamin K₁ is in some manner involved in phosphorylation. Menadione is inactive in this system but both it and phthiocol are able to augment markedly photosynthetic phosphorylation in chloroplasts (Arnon et al., 1955). Additional evidence is provided by the uncoupling action of Dicumarol, a vitamin K antagonist. Phosphorylation accompanying the oxidation of hydroquinones has been demonstrated in many systems, and a few examples will be mentioned. Menadiol is oxidized by an enzyme preparation from Azotobacter vinelandii and this is associated with the formation of some ATP, although the P:O ratio is low (Schils et al., 1960). Mitochondria similarly oxidize Q₆H-P with the generation of ATP, this being sensitive to antimycin A and 2,4-dinitrophenol, and it is likely that the ubiquinones participate directly in phosphorylation during electron transport (Gruber et al., 1963). The phosphorylation occurring during the oxidation of 3-methylmenadiol-P by mitochondria seems to involve the phosphorylated semiquinone intermediate, which was detected by electron-spin resonance (Bond and Mason, 1962). Oxidative phosphorylation by mycobacterial preparations is lost following ultraviolet irradiation, and this may be restored by vitamin K₁ (Brodie and Ballantine, 1960). Certain other naphthoquinones can restore electron transport but not phosphorylation. More indirect evidence suggests that α-tocopherylquinone may also participate in phosphorylations.

Various mechanisms have been proposed and most involve a phosphorylated hydroquinone intermediate; several reviews are available (Harrison, 1958; Brodie, 1961, 1964; Todd, 1960; Clark and Todd, 1960). The exact reactions whereby the phosphorylated intermediate is formed have not

\[
\begin{align*}
\text{Ubichromenol} & \\
\begin{array}{c}
\text{CH}_3 \\
\text{CH}_3 \\
\text{H}_2\text{C} \\
\text{CH}_2-\text{C}=\text{C}-\text{CH}_3 \\
\end{array} & \\
\begin{array}{c}
\text{CH}_3 \\
\text{CH}_3 \\
\text{O} \\
\end{array} & \\
\text{OH} & \\
\end{align*}
\]

been completely established, but Brodie (1964) since 1961 has accumulated much evidence that 6-chromanol derivatives are formed from both ubiquinones and vitamin K₁ by cyclization involving the γ-position of the side chain. Saturation of the phytol side chain of vitamin K₁ to give 2,3-dihydrophytyl-vitamin K₁ not only abolishes the ability to restore phospho-
Inhibition in irradiated preparations, but introduces an uncoupling activity (Brodie and Russell, 1963). The 6-chromanol-P derivative of vitamin K\(_1\), when added to a mycobacterial extract, cytochrome c, and a P-acceptor, causes the anaerobic reduction of cytochrome c, the formation of vitamin K\(_1\), and the appearance of ATP (Asano et al., 1962), and such chromanol derivatives have been detected during oxidative phosphorylation, including the 6-chromanol of ubiquinone. The detailed sequence of phosphate transfer has not been elucidated but possibly cyclization is necessary for the process and the retention of energy in the phosphate bonds. In any event, the effects of quinones on oxidative phosphorylation must be considered against a background of the widespread participation of various naturally occurring quinones and their derivatives in the transphosphorylation reactions.

Inhibition of Oxidative Phosphorylation

Four general mechanisms for the interference by exogenous quinones in phosphorylations may be readily suggested: (1) a competitive displacement of the endogenous quinones from their loci in the electron transport chain or their interaction sides, (2) a competitive acceptance of phosphate without the ability to transfer the phosphate to ADP, (3) a shunting of electron flow through nonphosphorylating pathways, and (4) a reaction with SH groups somehow involved in the transphosphorylations since other SH reagents have occasionally been shown to uncouple oxidative phosphorylation. We shall first discuss briefly some of the results obtained and then return to the problem of the mechanisms which may be involved.

Martius and Nitz-Litzow (1953) found phthiocol to be a very potent depressant of \(\beta\)-hydroxybutyrate oxidation by liver mitochondria, 0.0048 mM inhibiting oxygen uptake 61%; however, phosphorylation is reduced 100%, so that a degree of uncoupling was observed. Menadione acts similarly but is much less potent. A true uncoupling by menadione was noted in a particulate fraction from \textit{Micrococcus lysodeikticus} oxidizing NADH, the P:O ratio being reduced from 0.27 to 0.04, oxygen uptake being slightly increased (Ishikawa and Lehninger, 1962), and an even more striking uncoupling was reported in a preparation from \textit{E. coli}, lapachol lowering the P:O ratio from 1.1 to 0.2 and stimulating the respiration with malate as the substrate (Kashket and Brodie, 1963 a). Coenzyme Q analogs are occasionally efficient uncouplers. Jacobs and Crane (1960) divided these analogs into three groups on the basis of their action on liver mitochondria oxidizing succinate:

(I) \(Q_1\) and methyl-\(Q_6\): neither uncouple nor inhibit respiration

(II) \(Q_0\), 6-Cl-\(Q_0\), and 6-Br-\(Q_6\): uncouple and inhibit respiration

(III) \(Q_2\) and \(Q_5\): uncouple and slightly stimulate respiration
Schulz and Goss (1956) demonstrated an *in vivo* depression of the incorporation of P$_i^{32}$ into ATP in liver and heart following the intraperitoneal injection of menadione at 110–120 mg/kg, and felt that this indicated an uncoupling action which might be responsible for the toxicity of menadione. Actually no evidence for uncoupling was presented, and no reduction in the P:O ratio of liver mitochondria obtained from poisoned animals was noted. Parmar and Lowenthal (1962) also could find no uncoupling in liver mitochondria after hemorrhagic doses of the vitamin K$_1$ antagonist, 2-chloro-3-phytyl-1,4-naphthoquinone, so it appears that the reduction of prothrombin synthesis is not related to uncoupling, although one must admit the possibility that the antagonist was lost during the preparation of the mitochondria. There has been no clear-cut evidence for uncoupling by the simpler quinones, such as *p*-benzoquinone and 1,4-naphthoquinone, and indeed Ernster *et al.* (1963) detected no significant effects of these on the P:O ratio of liver mitochondria with glutamate as the substrate. The ATP:P$_i$ exchange reaction in liver mitochondria is slightly stimulated by menadione at 0.00001–0.0001 mM and inhibited 10% at 0.01 mM, 24% at 0.1 mM, and 58% at 1 mM (Dallam and Hamilton, 1964). The 2,4-dinitrophenol-activated ATPase activity is also inhibited.

Despite the fact that menadiol is oxidized with a P:O ratio of 0.2 by an enzyme system from *Azotobacter vinelandii*, it uncouples the phosphorylation associated with the oxidation of NADH (Schils *et al.*, 1960). It is interesting that lapachol competitively inhibits the phosphorylation during menadiol oxidation, but this does not necessarily indicate that a competitive action is exerted on the normal system. However, lapachol competitively prevents the reactivation of the irradiated mycobacterial system by vitamin K$_1$ (Brodie and Ballantine, 1960). The strong inhibitions exerted by Q$_0$ and 6-Br-Q$_0$, discussed in the preceding paragraph, are not competitive since they cannot be reversed by active coenzyme Q analogs (Smith and Lester, 1961). Thus there is so far very meager evidence for truly competitive inhibition by the quinones. Several have suggested an electron-shunting mechanism and certainly some nonphosphorylating pathways are known, as the menadione bypass of vitamin K$_1$ (Brodie, 1964), and it is likely that this is important in some instances, but again we have little direct evidence. Smith and Lester (1961) suggested that the uncoupling action of Q$_0$ and 6-Br-Q$_0$ is at least partially due to shunting electrons around a phosphorylating step, but they also favored the idea that reaction with SH groups may contribute to this, as did Jacobs and Crane (1960). It is clear that our knowledge of this aspect of the actions of quinones is rudimentary and much remains to be done in this important field.
EFFECTS ON GLYCOLYSIS

Quinones frequently inhibit glycolysis strongly and it is likely that this plays an important role in their actions on certain organisms and tissues. Some results are summarized in Table 5-4 and certain tentative conclusions may be drawn: (1) the glycolytic inhibition is occasionally very potent; (2) there is a good deal of variation in response between different organisms and tissues; (3) the naphthoquinones are generally more potent inhibitors than the benzoquinones; (4) there is often a diphasic effect with stimulation at lower concentrations; and (5) aerobic glycolysis seems to be more sensitive than anaerobic glycolysis to the quinones, although this may be due mainly to the fact that the inhibitory form is the quinone and not the hydroquinone. Aerobic lactate formation may be decreased, unaffected, or increased, probably depending on the balance of action on the glycolytic and oxidative pathways, and on the magnitude of the Pasteur-Crabbtree interactions in the particular tissue. Over-all glucose utilization is strongly depressed both anaerobically and aerobically. The inhibition of glucose uptake by Schistosoma was found to be greater than the inhibition of aerobic glycolysis (Bueding, 1950; Bueding et al., 1947), and the utilization of glucose by Fusarium is markedly reduced by 1,4-naphthoquinone, definite effects being exerted at 0.0063 mM (Maselli and Nord, 1952). It is interesting that injections of menadione in rats lead to a mean fall in liver glycogen from 15.3 mg/g to 0.9 mg/g in 20-50 min (Schulz and Goss, 1956), but it is not known whether this is due to a block in glycogen formation or to a stimulation of its utilization by some mechanism. Glycogen formation in yeast in strongly depressed by various quinones, the most potent, 1,4-naphthoquinone, inhibiting 55% at 0.01 mM (Hoffmann-Ostenhof and Kriz, 1949 b), but even here it is not certain if this may not be partly due to increased breakdown.

The major site for the glycolytic inhibition has not been definitely established. Inspection of Table 5-2 shows only two enzymes of the EM pathway, hexokinase and 3-phosphoglyceraldehyde dehydrogenase, to have been directly studied and both to be readily inhibited. Some analysis of the site of action was undertaken by Meyerhof and Randall (1918) in brain extracts. The inhibition of glycolysis by p-benzoquinone and various 1,2 naphthoquinones is greater with glucose than with fructose-1,6-diP as the substrate, and they concluded that at least part of the glycolytic inhibition must be on hexokinase. However, the inhibition when glucose-6-P is the substrate is also high so that perhaps some action on phosphohexokinase is suggested. Hochstein and Cohen (1960 b) reported different results in brain homogenates in that the inhibitions by p-benzoquinone and menadione are the same for both glucose and fructose-1,6-diP, but when 3-P-glycerate is the substrate the inhibition is definitely less, implicating the 3-phosphoglyceraldehyde dehydrogenase as the major site of attack.
<table>
<thead>
<tr>
<th>Organism or tissue</th>
<th>Conditions</th>
<th>Quinone</th>
<th>Concentration (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast</td>
<td>Anaerobic</td>
<td>p-Q</td>
<td>0.026</td>
<td>Stim 9</td>
<td>Hoffmann-Ostenhof and Kriz (1949 b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.26</td>
<td>Stim 11</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.6</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>TQ</td>
<td>0.026</td>
<td>Stim 14</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.26</td>
<td>Stim 23</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2.6</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,2-NQ</td>
<td>0.001</td>
<td>Stim 27</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1,4-NQ</td>
<td>0.01</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Phthiocol</td>
<td>0.026</td>
<td>Stim 7</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>0.26</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td><em>Trypanosoma equiperdum</em></td>
<td>Anaerobic</td>
<td>1,2-NQ-4-SO$_2^-$</td>
<td>0.036</td>
<td>6</td>
<td>Meyerhof and Randall (1948)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.072</td>
<td>28</td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
<td>0.18</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.36</td>
<td>66</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.72</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td><em>Balantidium coli</em></td>
<td>Anaerobic</td>
<td>MD</td>
<td>1</td>
<td>69</td>
<td>Agosin and von Brand (1953)</td>
</tr>
<tr>
<td><em>Schistosoma mansoni</em></td>
<td>Aerobic</td>
<td>p-Q</td>
<td>0.02</td>
<td>11</td>
<td>Bueding <em>et al.</em> (1947)</td>
</tr>
<tr>
<td>Compound</td>
<td>Aerobic</td>
<td>Anaerobic</td>
<td>Frog muscle</td>
<td>Rabbit leucocytes</td>
<td>Bull spermatazoa</td>
</tr>
<tr>
<td>--------------------------</td>
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<td>-----------</td>
<td>-------------</td>
<td>-------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>p-QH₂</td>
<td>0.2</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2-NQ</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,4-NQ</td>
<td>0.02</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MD</td>
<td>0.1</td>
<td>0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-CH₃O-MD</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lawsone</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic MD</td>
<td>0.02</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Cl-1,4-NQ</td>
<td>0.04</td>
<td>0.13</td>
<td></td>
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<tr>
<td>2,3-diCl-1,4-NQ</td>
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<td></td>
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<tr>
<td>2-NH₂-1,4-NQ</td>
<td>&gt;2</td>
<td>0.3</td>
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</tr>
<tr>
<td>3-CH₃-MD</td>
<td>&gt;0.8</td>
<td>4</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1,2-NQ-4-SO₃⁻</td>
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</tr>
<tr>
<td>Frog muscle Anaerobic</td>
<td></td>
<td></td>
<td>1,2-NQ-4-SO₃⁻</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>MD</td>
<td>0.1</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rabbit leucocytes Aerobic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MD</td>
<td>0.1</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bull spermatazoa Aerobic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-Q</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-QH₂</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Organism or tissue</th>
<th>Conditions</th>
<th>Quinone</th>
<th>Concentration (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse brain extract</td>
<td>Anaerobic</td>
<td>p-Q</td>
<td>0.055</td>
<td>80</td>
<td>Meyerhof and Randall (1948)</td>
</tr>
<tr>
<td>Mouse brain homogenate</td>
<td>Aerobic</td>
<td>p-Q</td>
<td>0.0056</td>
<td>21</td>
<td>Hochstein and Cohen (1960 b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-QH₂</td>
<td>0.45</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-QH₂</td>
<td>0.011</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Anaerobic</td>
<td>p-Q</td>
<td>0.0056</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>MD</td>
<td>0.28</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>Rat sarcoma slices</td>
<td>Aerobic</td>
<td>p-Q</td>
<td>1</td>
<td>0</td>
<td>Elliott and Baker (1935)</td>
</tr>
<tr>
<td>Ascites carcinoma</td>
<td>Anaerobic</td>
<td>p-Q</td>
<td>0.31</td>
<td>58</td>
<td>Holzer et al. (1956)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.62</td>
<td></td>
<td>65</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.25</td>
<td></td>
<td>92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aerobic</td>
<td>MD</td>
<td>0.02</td>
<td>56</td>
<td>Tiedemann et al. (1958)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.04</td>
<td></td>
<td>87</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>MD</td>
<td>0.04</td>
<td>34</td>
<td>Tiedemann and Risse (1960)</td>
</tr>
<tr>
<td></td>
<td>Aerobic</td>
<td>9,10-PAQ</td>
<td>0.01</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>
Tiedemann et al. (1958) reported that the inhibitions of ascites tumor cell aerobic glycolysis by 0.04 mM menadione are 87% with glucose, 57% with glucose-6-P, and 49% with fructose-1,6-diP as substrates, these results indicating significant inhibitions of hexokinase and some enzyme distal to fructose-1,6-diP (probably 3-phosphoglyceraldehyde dehydrogenase), with a minor action on phosphohexokinase. 9,10-Phenanthraquinone causes an accumulation of glucose-6-P (2×), fructose-1,6-diP (10×), and 3-phosphoglyceraldehyde (4–5×) in ascites cells (Tiedemann and Risse, 1960), which might be taken as indicating inhibitions of phosphohexokinase and 3-phosphoglyceraldehyde dehydrogenase, but it was also shown that this quinone rapidly reduces both ATP and NAD levels in glycolyzing cells so that a substantial part of the inhibition may be related to this rather than to a direct action on the enzymes. Holzer (1956) and Holzer et al. (1956) favored 3-phosphoglyceraldehyde dehydrogenase as the primary site of inhibition on the basis of comparative studies with a variety of carcinostatic quinones, and Cohen and Hochstein (1960) also prefer this site for the inhibition of brain glycolysis. On the other hand, Kiesow (1960 a) found that menadione rather potently reduces the uptake of 2-deoxyglucose by yeast, this being presumably a measure of hexokinase inhibition, and since the effects are about the same as on fermentation this enzyme was considered to be a major site of attack. Summarizing all of this evidence, one can conclude only that both hexokinase and 3-phosphoglyceraldehyde dehydrogenase are usually inhibited and represent the important glycolytic inhibition sites, with phosphohexokinase contributing to a minor extent, and that all of the inhibition may be not directly on these enzymes but also dependent on reduction of ATP and NAD levels. In this connection it is important to remember that hydrogen peroxide is a potent inhibitor of glycolysis and is formed aerobically during the oxidation of hydroquinones. Hochstein and Cohen (1960 a) pointed out that glycolysis in brain homogenates is very sensitive to quinones and that protection is afforded by supernatant fractions from other tissues, part of this protection being related to the catalase content. Pütter (1963) demonstrated that quinones increase the hydrogen peroxide levels in ascites tumor cells, and postulated that the falls in ATP and NAD could well be related to this. Inhibitions of anaerobic glycolysis would, of course, not be attributed to hydrogen peroxide.

The understanding of how naphthoquinones alter glucose metabolism in brain has recently been enlarged by the excellent studies of Hoskin using K+-stimulated guinea pig cortex slices. Menadiol-diP, which presumably is active by virtue of the menadione formed from it, was shown to alter the pattern of glucose metabolism in a characteristic way (see accompanying tabulation) (Hoskin, 1960 b). There is a slight stimulation of the respiration and scarcely any effect on the C$^{14}$O$_2$ derived from glucose-6-C$^{14}$, but the formation of C$^{14}$O$_2$ from glucose-1-C$^{14}$ is markedly augmented so
that the C-1/C-6 ratio rises, pointing to an activation of the pentose-P pathway. Menadione is about 100 times more potent than menadiol-diP

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Menadiol-diP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5 mM</td>
</tr>
<tr>
<td>O₂ uptake (µl/g)</td>
<td>3208</td>
<td>3650</td>
</tr>
<tr>
<td>CH₄O₂ from (%/g)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose-1-CH₁⁴</td>
<td>20.3</td>
<td>46.4</td>
</tr>
<tr>
<td>Glucose-6-CH₁⁴</td>
<td>18.4</td>
<td>14.8</td>
</tr>
<tr>
<td>C-1/C-6 ratio</td>
<td>1.10</td>
<td>3.13</td>
</tr>
</tbody>
</table>

and stimulates the pentose-P pathway maximally at 0.001 mM (Hoskin, 1960 a). The relative activities of various quinones are shown in the accompanying tabulation (Hoskin, 1962; Hoskin and von Eschen, 1963),

<table>
<thead>
<tr>
<th>Effective (at 0.01 mM)</th>
<th>Slightly effective</th>
<th>Ineffective (up to 0.05 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD</td>
<td>1,4-NQ</td>
<td>p-Q</td>
</tr>
<tr>
<td>3-CH₃-MD</td>
<td>Juglone</td>
<td>TQ</td>
</tr>
<tr>
<td>Tri-CH₃-p-Q</td>
<td>Naphthazarin</td>
<td>p-XQ</td>
</tr>
<tr>
<td>Plumbagin</td>
<td></td>
<td>Phthiole</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lawlsone</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Q₁₀</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vitamin K₁</td>
</tr>
</tbody>
</table>

from which it may be concluded that this activity is dependent on a 2-CH₃ group, and either CH₃ groups at positions 5 and 6 or a ring on that side. Long side chains, as in Q₁₀ and vitamin K₁, were thought to possibly prevent access to the enzymes involved. Using soluble brain preparations, Hoskin and von Eschen (1963) have shown that the active quinones stimulate the metabolism of glucose-6-P if NADP is added, and also stimulate the oxidation of NADPH. Some stimulation with menadione occurs at 0.001 mM, is maximal at 0.01 mM, and remains constant as the concentration is raised to 1 mM, showing that even at these high concentrations there is no inhibition of glucose-6-P metabolism. The mechanism of the stimulation is apparently a rapid regeneration of NADP, with autoxidation of the menadiol formed, so that glucose is fed into the pentose-P pathway at an accelerated rate. Some of the less effective quinones may be inactivated by reactions with proteins.
Menadione has similar effects on the pattern of glucose metabolism in guinea pig nonphagocytosing polymorphonuclear leucocytes (see accompanying tabulation), in that $\text{C}^{14}\text{O}_2$ arising from glucose-1-$\text{C}^{14}$ is markedly stimulated while that from glucose-6-$\text{C}^{14}$ is unaffected, indicating an activation of the pentose-P pathway (Iyer et al., 1961). However, here, in contrast to brain, the pentose-P pathway normally is to some extent active, and there is a much greater elevation of respiration. The effects of menadione mimic those changes occurring when phagocytosis is initiated, so that menadione has relatively little action on glucose metabolism in phagocytosing leucocytes.

The thorough study of the effects of 104 quinones on aerobic glycolysis in *Schistosoma mansoni* by Bueding and Peters (1951) presents interesting possibilities of correlating structure with inhibitory activity, but certain factors make accurate comparison difficult. First, the degree of penetration into the worms may limit the actions of some of the quinones. This may be responsible for the low activities of the sulphonates of both the 1,2- and 1,4-naphthoquinones, but could also play a role in determining the potencies of some of the nonionic quinones. Second, aerobic glycolysis is measured and this is dependent on several pathways and many factors, so that the actions of all these quinones cannot be attributed necessarily to a common mechanism. Third, the metabolism of this organism is unusual; e.g., glucose is transformed mainly to lactate aerobically, since the systems for pyruvate oxidation are deficient, and changing to anaerobic conditions does not alter the rate of glucose utilization or lactate formation. Quite possibly the mechanisms of inhibition in these worms are different from those in mammalian tissues. Bueding and Peters pointed out that there is no correlation between the glycolytic inhibitions here and the inhibitions of respiration or glycolysis in other organisms or tissues. There is certainly no good evidence that the inhibition in *Schistosoma* is related basically to reaction with SH groups. Although addition of a methyl group to menadione in the 3-position markedly reduces the activity, some of the most potent
inhibitors are substituted in both 2- and 3-positions, e. g., the 3-thioalkyl derivatives of menadione, which are usually more inhibitory than menadione. It should be noted that 1,2-naphthoquinone is also a very potent inhibitor and yet does not readily react with SH groups.

EFFECTS ON RESPIRATION

The responses of respiration to the quinones are variable and complex, and depend on the species or tissue used, the type of quinone, and the conditions of the experiment. It is probably safe to state that in all the instances of respiratory inhibition reported the site or sites of action are unknown. Surprisingly little is known of the effects of the quinones on the oxidation of pyruvate or the operation of the cycle, and this impedes our understanding of respiratory inhibitions since the cycle is the most likely general site for the action. There are actually so many ways in which the quinones could alter oxygen uptake that a detailed discussion of the effects on respiration would be meaningless without more information on these mechanisms. We shall therefore confine our discussion to the most general topics and attempt to illustrate briefly the types of effect commonly observed.

One characteristic response of the respiration to the quinones is immediately evident; this is the tendency for the respiration to be increased, especially at low quinone concentrations, the action being biphasic if a sufficiently wide range of concentrations is used. Hydroquinones often stimulate oxygen uptake simply by acting as substrates, but we have seen that many quinones can elevate electron transport through oxidation-reduction reactions in various steps of the sequence, this not necessarily involving direct effects on the participating enzymes. A few examples of diphasic response are given in the accompanying tabulation.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Quinone</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast</td>
<td>Thymoquinone</td>
<td>Flaig and de Jong (1960 b)</td>
</tr>
<tr>
<td>Chlorella</td>
<td>MD</td>
<td>Gaffron (1945)</td>
</tr>
<tr>
<td></td>
<td>Phthiocol</td>
<td></td>
</tr>
<tr>
<td>Schistosoma</td>
<td>p-Q</td>
<td>Bueding et al. (1947)</td>
</tr>
<tr>
<td></td>
<td>MD</td>
<td></td>
</tr>
<tr>
<td>Bull spermatozoa</td>
<td>p-QH₂</td>
<td>Lardy and Phillips (1943 a)</td>
</tr>
<tr>
<td>Kidney slices</td>
<td>p-Q</td>
<td>Kisch and Leibowitz (1930)</td>
</tr>
<tr>
<td></td>
<td>o-Q</td>
<td></td>
</tr>
<tr>
<td>Liver slices</td>
<td>Phthiocol</td>
<td>Supniewski et al. (1936)</td>
</tr>
<tr>
<td>Ascites carcinoma</td>
<td>MDH₂-diP</td>
<td>Chipperfield and Marrian (1962)</td>
</tr>
</tbody>
</table>
example, which is quite typical, is illustrated in Fig. 5-4. In other cases only respiratory stimulation has been reported, although it is likely that inhibition would have been noted at higher quinone concentrations. We have discussed the augmentation of respiration in brain (Hoskin, 1960 b) and leucocytes (Iyer et al., 1961) by menadione. The effects on erythrocyte respiration apparently involve special mechanisms and are not completely understood. Friedheim (1934) noted that juglone and lawsone at 1 mM increase the oxygen uptake of rabbit erythrocytes 5- to 6-fold in the presence of glucose, and claimed that the effect is not linked to the hemoglobin redox system since he could detect no formation of methemoglobin. Lovell and Pranker (1961) pointed out that the small respiration of erythrocytes is probably related to the oxidation of hemoglobin, and then showed quite conclusively that 1,4-naphthoquinone and menadione cause a rapid oxidation of hemoglobin to methemoglobin, and a corresponding augmentation of the oxygen uptake. Harley and Robin (1963) also showed that 1,2-naphthoquinone, 1,4-naphthoquinone, and menadione catalyze the conversion of hemoglobin to methemoglobin; e. g., at a molar ratio of 1,2-naphthoquinone to heme of 1:32, 60% of the hemoglobin is oxidized in 4 hr. The reaction is apparently dependent on NADPH and is thereby related to the operation of the pentose-P pathway, which can normally provide
reductive energy for the restoration of hemoglobin. Supniewski et al. (1936) reported an odd biphasic action, difficult to explain, in that low concentrations (around 0.05 mM) of phthiocol depress the respiration of mouse erythrocytes while higher concentrations (0.5–2.6 mM) progressively stimulate. Actually these mechanisms involving hemoglobin may well occur in other cells with various substances taking the place of hemoglobin. Stimulation of respiration probably depends to a great extent on the state of the cells and particularly on how active the respiration is. For example, several investigators have found yeast respiration to be inhibited readily by \( p \)-benzoquinone, but Lejhanec et al. (1931) observed quite marked stimulation at 0.018 mM, the oxygen uptake sometimes increasing as much as 2- to 10-fold, and the explanation for this discrepancy may well be that the respiratory rate was for some reason rather low in the experiments of Lejhanec. *Chlorella* respiration in the presence of glucose is inhibited by phthiocol at 0.1 mM, but when the cells are starved and the rate initially low the respiration may be stimulated as much as 15-fold by phthiocol (Gaffron, 1945), and the respiration of substrate-depleted yeast is more apt to be stimulated by low concentrations of various quinones than is the respiration of fresh yeast (Flaig and de Jong, 1960 a).

Comparison of the effects of the quinones on respiration and glycolysis, or on endogenous and glucose respiration, shows that no general rules can be formulated and many factors may be involved. One important factor is the levels of ATP and ADP. Phenanthraquinone at 0.01 mM progressively depresses the endogenous respiration of ascites tumor cells, but in the presence of glucose there is some initial stimulation and the respiration then returns to near-normal levels (Tiedemann and Risse, 1960). In cells utilizing glucose the quinone causes a piling up of hexose and triose phosphates, with a consequent fall of ATP and rise of ADP, the latter accounting for the acceleration of respiration, while in the absence of glucose this cannot occur, the ATP level is unchanged, and respiration is gradually depressed. This mechanism may be involved in the suppression of the Pasteur effect in yeast by thymoquinone (Flaig and de Jong, 1960 b). The ratio of the glucose utilization anaerobically and aerobically, which is near 2 in normal yeast, is progressively reduced to almost 1 by increasing concentrations of thymoquinone. Since 2,4-dinitrophenol also exerts this effect, an uncoupling action by the quinone was considered, but the effects of the two substances on polysaccharide synthesis are different and it is likely that the major action of the quinone is not uncoupling.

Another important factor in the over-all effects of the quinones on carbohydrate metabolism and respiration is the relative sensitivities of the EM pathway, the lactate or alcohol dehydrogenases, and the enzymes for the disposal of pyruvate. These relationships could be better understood if there were more data on the alterations of keto acid levels occurring
during the action of the quinones. There is a fairly marked augmentation of pyruvate accumulation in *Fusarium* by low concentrations of 1,4-naphthoquinone (Fig. 5-5) but these measurements were made over many days and do not reflect the immediate changes, and furthermore this organism has apparently an unusual pattern of metabolism (Maselli and Nord, 1952). Indeed, the early effect of the quinone may be a block of the EM pathway, since the effect on pyruvate accumulation is delayed. The keto acid level rises somewhat in yeast exposed to 0.001 mM toluquinone, but as the quinone concentration is increased this effect is reversed and the levels fall (Flaig and de Jong, 1960 a). It is likely that the keto acid elevation is due not to an inhibition of utilization but to a stimulation of formation from glucose. Certainly pyruvate decarboxylation in yeast is not particularly sensitive to the quinones, and is not inhibited at all by 0.1 mM toluquinone (Hoffmann-Ostenhof and Kriz, 1949 b). Indeed, the oxidation of pyruvate and the formation of $\text{C}^{14}\text{O}_2$ from pyruvate-$3\text{-C}^{14}$ in sheep thyroid slices are stimulated by menadiol-diP at 0.09 mM (Dumont, 1962).

One must constantly remember that the quinones do not compose a homogeneous group with respect to their metabolic effects, and that results obtained on one quinone do not necessarily apply to others. In other words, one should avoid as much as possible saying that the "quinones" do this or that, this implying certain common actions. Thus the interesting effects of phenanthraquinone reported by Tiedemann and Risse (1960) and dis-

![Fig. 5-5. Effects of 1,4-NQ on pyruvate accumulation in cultures of *Fusarium*. (Data from Maselli and Nord, 1952.)](image-url)
cussed above probably do not parallel the effects of the simpler quinones. For example, Shacter (1956) found only a progressive depression of the respiration of ascites tumor cells by p-benzoquinone in the presence of glucose, instead of the stimulation seen with phenanthraquinone. It is doubtful if any actions of phenanthraquinone are related to SH groups. The purposes of the investigations in which many types of quinone have been compared for their effects on glycolysis or respiration — e.g., the reports using Schistosoma (Bueding and Peters, 1951), Australorbis (von Brand et al., 1949), or Photobacterium (Spruit and Schuiling, 1945) — did not require a metabolic analysis of the different types of action, so that unfortunately we have no information on the relative importance of the various possible sites of inhibition.

A few interesting and surprising results obtained in studies on the effects of quinones on respiration will be mentioned briefly. The pyruvate decarboxylase of Monilinia fructicola is quite sensitive to several quinones (Table 5-2) and the inhibition of spore germination generally parallels the enzyme effects, so that it was suggested that the site of the inhibition is the decarboxylase (Foote et al., 1949). However, these quinones scarcely affect respiration and may even stimulate it moderately. Is the respiration of this organism independent of pyruvate, and, if the germination depends on the decarboxylase, how is pyruvate metabolized? Unexpected results on the reversibility of the effects of p-benzoquinone on the respiration and motility of bull spermatozoa were reported by Lardy and Philips (1943 a). The quinone was found to produce an irreversible inhibition, whereas the hydroquinone inhibition is reversible. One might superficially attribute the hydroquinone effects to the quinone formed from it by oxidation, but this does not seem to be the case, since if this were the mechanism the inhibition would also be irreversible. The quinone may well inhibit by a reaction with SH groups, but how does the hydroquinone inhibit? Arsenite inhibits the respiration of bone marrow, leucocytes, and lymphogenous and myelogenous leukemic tumors, and simultaneously there is some increase in aerobic lactate formation (Warren, 1943). This would usually be attributed to a block of pyruvate oxidation. Menadione is able to counteract these effects of arsenite, restoring the respiration and lowering the aerobic glycolysis, although there is no antagonism of the effects on leucocyte motility or cytological changes. Since methylene blue and thionine are also able to counteract the arsenite effects, it was postulated that menadione action is in some way related to its redox potential, this being substantiated by the fact that phthiocol, with a much lower potential, is without effect. The question here is: How could menadione initiate or activate some oxidative sequence which would bypass or overcome the arsenite effect? Whatever the mechanism, it is always interesting when two inhibitors counteract each other’s effects, and a more detailed study of this particular case might
provide useful information not only on the subject of multi-inhibition but also on the individual actions of the two types of inhibitor.

**EFFECTS ON VARIOUS METABOLIC SYSTEMS**

Very little is known of the effects of the quinones on lipid, protein, or nucleic acid metabolism. Maselli and Nord (1949) claimed that naphthoquinones suppress lipid formation in *Fusarium lini* at very low concentrations (see accompanying tabulation), and further alter the lipid compo-

<table>
<thead>
<tr>
<th>Quinone</th>
<th>1.4-Naphthoquinone</th>
<th>Menadione</th>
<th>Phthiocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0.006 mM)</td>
<td>- 20</td>
<td>- 46</td>
<td>- 57</td>
</tr>
<tr>
<td>% Change in:</td>
<td>- 15</td>
<td>- 20</td>
<td>+ 1</td>
</tr>
<tr>
<td>Mycelial weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Total lipid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Sterols in fat</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

sition and increase fat desaturation. Phthiocol apparently acts somewhat differently because, although it suppresses growth potently and reduces the sterol content, it does not inhibit total lipid synthesis. In results like this it is difficult to know if the lipid changes are due to direct action on lipid metabolism or are secondary to growth depression. Maselli and Nord (1952) believe that the naphthoquinones inhibit both the glucose utilization and the metabolism of pyruvate to lipid; when acetate is present the inhibitions are less. In yeast the picture is quite different, various quinones either not effecting lipid synthesis or stimulating it somewhat, the stimulation being mainly in the sterol fraction (Hoffmann-Ostenhof and Kriz, 1949 a, b). Even less is known about amino acid metabolism. *p*-Benzoquinone has been reported to accelerate the oxidative deamination of amino acids in cress roots (Flaig and Reinemund, 1955), and *p*-benzohydroquinone reduces hair color, skin pigmentation, and melanin formation, presumably blocking the oxidation of tyrosine (Denton et al., 1952). *p*-Benzoquinone and 1,4-naphthoquinone markedly stimulate the “incorporation” of tyrosine-2-C14 into the proteins of liver homogenates (Kamin et al., 1957). Actually it is not tyrosine but a melanin-like substance that is incorporated, and much of the action is nonenzymic, occurring in the absence of the homogenate. Protein synthesis in *Pseudomonas aeruginosa* is quite potently inhibited by both *p*-benzoquinone and *p*-benzohydroquinone, since the formation of the adaptive enzyme for benzoate oxidation is depressed 76% by 0.09 mM (Bernheim, 1954). However, it is not known whether the site of
inhibition is directly on protein synthesis or is a simple reduction in the energy-supplying reactions. Menadione does not alter the incorporation of formate or glycine into the purines of the acid-soluble nucleotides or of DNA, but reduces the uptake into RNA purines, and this is accompanied by an increase in the pyrimidines at the expense of the purines (Marrian, 1959). It was thought that this might explain the radiosensitizing action, since X-irradiation interferes predominantly with DNA synthesis.

**Thyroid Metabolism**

Diiodotyrosine and thyroxine formation in thyroid slices is inhibited around 80% by 1 mM \( p \)-benzohydroquinone (Taurog et al., 1945). Since the monomethyl ether of \( p \)-benzohydroquinone inhibits similarly, and inasmuch as several compounds related structurally to thyroxine also inhibit it is likely that the inhibition is not directly related to a quinone structure. However, it was suggested that the ease of oxidation has something to do with the inhibition and that the blocked step is probably an oxidative one. The uptake of \( {I}^{131} \) into thyroid slices is depressed 60–90% by 1 mM \( p \)-benzohydroquinone, menadione, menadiol-diP, and hydrolapachol (Slingerland, 1955). The site of action is presumably on the uptake process, since the incorporation of \( {I}^{131} \) into the proteins of subcellular fractions of thyroid is quite markedly stimulated by several quinones at 0.5 mM (see accompanying tabulation), although lawsone and phthiocol are inhibitory

<table>
<thead>
<tr>
<th>Quinone</th>
<th>% of total ( {I}^{131} ) in proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>4.0</td>
</tr>
<tr>
<td>Lapachol</td>
<td>5.0</td>
</tr>
<tr>
<td>Vitamin K(_1)</td>
<td>5.5</td>
</tr>
<tr>
<td>1,2-Naphthoquinone</td>
<td>30.2</td>
</tr>
<tr>
<td>1,4-Naphthoquinone</td>
<td>33.3</td>
</tr>
<tr>
<td>Juglone</td>
<td>33.8</td>
</tr>
<tr>
<td>Menadione</td>
<td>52.4</td>
</tr>
</tbody>
</table>

(Tong and Chaikoff, 1960, 1961). There is also some stimulation of \( {I}^{131} \) incorporation into pepsin and seralbumin nonenzymically, and it is likely that the mechanism involves the oxidation of the iodide to \( I_3^- \), HOI, or \( IOH_2^+ \) prior to the incorporation. Williams (1961) also noted that menadione at 0.03–0.1 mM increases the amount of \( {I}^{131} \) bound in thyroid slices. The situation here is probably complex and there are several ways in which the quinones can act. It is not known if the effects on \( {I}^{131} \) binding are related to the formation of thyroxine.
EFFECTS ON VARIOUS METABOLIC SYSTEMS

Photosynthesis

The photochemical release of oxygen in chloroplast suspensions (Hill reaction) is classically demonstrated with \( p \)-benzoquinone as the hydrogen acceptor, but a number of other quinones with values for \( E'_0 \) between

\[
p-Q + H_2O \rightarrow p-QH_2 + 1/2 O_2
\]

- 0.1 and + 0.44 V can also function in this reaction, as can various non-quinonoid substances such as NADP. A new type of quinone has recently been found to occur in chloroplasts and is able to restore the Hill reaction in extracted chloroplasts; this is the plastoquinone series (Bishop, 1960). The plastoquinones differ from the ubiquinones only in lacking the ring

![Plastoquinones](image)

methyl group, and having methyl groups instead of methoxy groups. It has been postulated that the plastoquinones may be reduced directly following the photoactivation of chlorophyll and function by transferring hydrogen atoms to NADP, perhaps also being involved in photophosphorylation. Possibly the artificial quinones previously used in the Hill reaction function as the natural plastoquinones. The discovery of these new quinones enlarges the interest in the actions of exogenous quinones on photosynthesis and opens up interesting possibilities for inhibitory mechanisms.

Photosynthesis in Chlorella is quite potently inhibited by menadione and phthiocoll, while at the same time respiration may be stimulated or unaffected (Gaffron, 1945). In general, photosynthesis is much more sensitive than respiration to the quinones. The chelating or uncoupling mechanisms suggested by Gaffron are unlikely explanations. When \( p \)-benzoquinone is used as a Hill reagent, the ability of the cells to fix \( CO_2 \) in photosynthesis is entirely lost (Clendenning and Ehrmantraut, 1950), so that the inhibitory action must be exerted on the later nonphotochemical steps. High concentrations of \( p \)-benzoquinone will actually inhibit photochemical oxygen release or the Hill reaction, but the usual concentrations can block photosynthesis without affecting oxygen evolution or respiration. Despite the fact that menadione and some other naphthoquinones can be used as Hill reagents, they can occasionally inhibit oxygen evolution, as they do in Elodea (Schopfer and Grob, 1949 a). \( p \)-Benzoquinone, duroquinone, and
choloranil can quench the fluorescence of chlorophyll and other porphyrins, but the concentrations required (3.4–11.6 mM) make them unlikely to be of significance in photosynthetic inhibition (Livingston and Ke, 1950; Livingston et al., 1952).

One might postulate that exogenous quinones reoxidize reduced plastoquinones and thus divert the electron flow to NADP:

\[
\begin{align*}
\text{Q} & \rightarrow \text{H}_2\text{O} \rightarrow \text{PQ} \rightarrow \text{FAD(?)} \rightarrow \text{NADP}
\end{align*}
\]

the reduction of which is necessary for the operation of the photosynthetic carbon cycle, and perhaps also prevent the phosphorylation occurring during the normal electron transfer. Miyachi et al. (1955) suggested a similar scheme but assumed a substance, R, formed in the photochemical reaction and necessary for CO2 fixation; inhibiting quinones oxidize R so that it cannot function. The plastoquinones could be visualized as R. Higher concentrations of quinones would irreversibly inactivate enzyme systems in addition to this oxidation of reduced plastoquinones.

**Bioluminescence**

The effects of the quinones on bioluminescence have been studied more thoroughly than most other metabolic systems because for many years it was thought a naphthoquinone might be involved in the normal production of light. Harvey (1929) showed that bacterial luminescence is inhibited by p-benzoquinone, p-xyloquinone, 1,2-naphthoquinone, 1,4-naphthoquinone, and 2-Cl-1,4-naphthoquinone, and attributed this to a direct oxidation of luciferin, since anthraquinone-2-sulfonate and anthraquinone-2,6-disulfonate do not inhibit and have very low redox potentials. However, these latter two substances are not ideal substances for comparison, since anthraquinones are often abnormally reactive and the sulfonate groups might well interfere with their penetration. p-Benzolquinone is not a very potent inhibitor and yet has a high redox potential, as shown in the study of Spruit and Schuiling (1945), who thought that luciferin might be a 1,4-naphthoquinone and hence examined several quinones in the hope of competitively inhibiting the reaction (see accompanying tabulation). Luminescence is always more sensitive than respiration, and this is evident in the curves of Fig. 5-4. Bacterial luciferin, which is now considered to be a complex of FMNH2 and a long-chain aldehyde, was assigned an \( E'_0 \) of \(-50\) mv, so it could be readily oxidized by most of the quinones studied. Since cyanide can counteract the inhibition, it was postulated that the hydroquinones must be reoxidized through the cytochrome system in order that the luciferin be kept oxidized. The specificity of quinones for the inhibition of bacterial luminescence was stressed by Rake et al. (1943), who
showed that it requires 500 times the concentration of toluquinone to inhibit streptococcal growth compared to inhibition of *Achromobacter fischeri* light emission, whereas various antibiotics do not show this specificity and

<table>
<thead>
<tr>
<th>Quinone</th>
<th>( E_0' ) (mv)</th>
<th>(1)(_{20}) for:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Luminescence</td>
</tr>
<tr>
<td>p-Benzoquinone</td>
<td>+ 284</td>
<td>4</td>
</tr>
<tr>
<td>1,2-Naphthoquinone</td>
<td>+ 127</td>
<td>0.032</td>
</tr>
<tr>
<td>1,4-Naphthoquinone</td>
<td>+ 70</td>
<td>0.005</td>
</tr>
<tr>
<td>Juglone</td>
<td>+ 33</td>
<td>0.007</td>
</tr>
<tr>
<td>Menadione</td>
<td>+ 20</td>
<td>0.005</td>
</tr>
<tr>
<td>Lawsons</td>
<td>− 139</td>
<td>0.2</td>
</tr>
<tr>
<td>Phthiocol</td>
<td>− 172</td>
<td>0.12</td>
</tr>
</tbody>
</table>

usually inhibit growth more effectively. Bacterial extracts luminesce when NAD is added and this is appreciably inhibited by 1,2-naphthoquinone and menadione at 0.0001 mM, around 50% at 0.01 mM, and completely at 0.1 mM (Strehler and Cornier, 1953). This could be interpreted either as an oxidation of luciferin or a block between NAD and luciferin.

McElroy and Kipnis (1947) objected to the luciferin oxidation mechanism on the basis that respiration is also quite well inhibited by the quinones and that cyanide does not reverse the inhibition when glucose is present — arguments which do not seem to be completely valid — and preferred to assume that luciferin is reduced by some factor X between cytochrome b and cytochrome c, luciferin actually competing with the cytochrome c for electrons. The naphthoquinones are assumed to act between cytochrome b and X, thereby inhibiting both luminescence and respiration; the effect on the latter may be less because another pathway for glucose oxidation exists. The modern scheme of bacterial luminescence (see page I-850) involves NAD in the reduction of FMN to FMNH\(_2\), which reacts with the aldehyde and the enzyme to form an excited complex, and it could well be that the quinones oxidize FMNH\(_2\) to prevent the formation of this complex, but no recent work has been done on this point. It would also be interesting to know if luciferase is inhibited by the quinones. The luciferase of *Renilla reniformis* is not inhibited at all by menadione at 0.1 mM, but neither is the light emission, and thus the luminescent system in the sea pansy must be quite different from that in bacteria (Cornier, 1960). Likewise, the luciferase of *Odontosyllis phosphorea*, a polychaete annelid, is inhibited only 15% by 0.5 mM menadione (Shimomura *et al.*, 1963).
EFFECTS ON TISSUE FUNCTIONS

The actions of the quinones on tissues are quite complex in most instances, as would be expected from the many possible mechanisms by which these substances can alter metabolism, but nevertheless some interesting results have been reported and should stimulate further investigation along certain lines which may be evident in the following discussion.

Skeletal Muscle and the Neuromuscular Junction

Baglioni (1905) showed that 0.23 mM 1-phenanthrolinquinone applied directly to frog muscle causes clonic spasms, and 1-phenanthrolinquinone, which is readily oxidized, is almost as active, whereas phenol, which is not so oxidized, is inactive. The question arose as to the necessity for the diphenols to be oxidized before exerting their characteristic toxic actions on tissues; if this is the case, the activity would depend on the rate at which the diphenol is oxidized under the experimental conditions. Labes (1929 a) undertook to settle this question by the application of 0.9 mM 1-phenanthrolinquinone to frog gastrocnemius aerobically and anaerobically. The muscle went into contracture in 15 min in the presence of oxygen, while in the absence of oxygen it shortened much more slowly and to a lesser degree. Furthermore, altering the pH gave results indicating that these effects on muscle to a large extent depend on the rapidity of hydroquinone oxidation, the activity increasing with the pH. Thus the more active form appears to be the quinone. Hinteregger (1930) confirmed and extended these observations. 1-Phenanthrolinquinone was found to act rapidly on frog muscle, quinhydrone more slowly, and 1-phenanthrolinquinone still more slowly. Increasing the pH from 5.8, where there is no action of the hydroquinone, to 8.5 markedly increases the effect, while oxidation of the hydroquinone catalytically with Mn++ also accelerates its action. Muscles obtained from hyperthyroid frogs are more susceptible to the effects of 1-phenanthrolinquinone, which might be interpreted as due to the more rapid oxidation to the quinone in such muscle, or, as Hinteregger also suggested, to an increased permeability, which could not be experimentally demonstrated. On the other hand, it is quite possible that muscles with a higher metabolic rate might go into contracture faster, this being independent of hydroquinone oxidation or permeability. It is likely that both the quinone and hydroquinone forms exert effects on muscle. Sterin (1935) found that resorcinol, which cannot be oxidized directly to a quinone, produces convulsive twitches of the gastrocnemius muscle, an action very similar to that by 1-phenanthrolinquinone. 1-Phenanthrolinquinone, on the other hand, tends to produce contracture, so that somewhat different actions may be associated with the two forms. It is tempting to relate the quinone-induced contracture to reaction with SH groups and to compare it with the Lundsgaard effect.
obtained with iodoacetate, as did Lecomte and Fischer (1948), who found that both p-benzoquinone and 2,6-dimethoxy-p-benzoquinone at 0.092 mM cause a slow irreversible contracture of the frog rectus abdominis muscle. It is interesting, but unexplained, that 2-hydroxy-1,4-naphthoquinone causes a reversible contracture. No studies of the metabolic changes associated with the actions of the quinones on intact muscle have been made, but several workers have noted that the respiration of muscle, more than most tissues, is often stimulated by both benzo- and naphthoquinones. One must also consider the possibility that at least part of the action is by hydrogen peroxide formed in the reoxidation of the hydroquinones, this leading to a glycolytic block.

The neuromuscular junction appears to be fairly sensitive to the quinones, which was indicated in the early work of Labes (1929 a) who found the indirect excitability through nerve stimulation to fail much earlier than direct excitability of the muscle during exposure to p-benzoquinone aerobically. The twitches are abolished by treatment of the muscle with curare or increasing the Ca++ concentration 4- to 6-fold (Sterin, 1935), indicating that the effect is related to cholinergic mechanisms at the end-plate. Hydroquinones are able to stimulate contractures in certain nerve-muscle preparations. Thus Sterin (1938) found p-benzoquinone to augment contractions in frog muscle fatigued by stimulation through its nerve, and Tagaki (1939) found both p-benzoquinone and catechol at low concentrations (0.002–0.009 mM) to increase the contractions of toad gastrocnemius stimulated through the sciatic nerve. Indeed, these hydroquinones have the ability to antagonize the neuromuscular block by curare, as shown by Mogey and Young (1949) in the rat phrenic nerve-diaphragm preparation. Such an action is most readily interpreted by an inhibition of cholinesterase, but they found brain and serum cholinesterase to be inhibited very weakly by both p-benzoquinone and catechol. Reaction with tubocurarine is unlikely, so the mechanism was unexplained. An inhibition of cholinesterase cannot be eliminated because, first, muscle cholinesterase was not tested and, second, inhibition by hydroquinones might occur more readily in the intact muscle due to higher levels of the corresponding quinone. Torda and Wolff (1946 a) indeed found p-benzo-hydroquinone to increase the response of frog muscle to acetylcholine, while simultaneously there is a decreased contraction from K+, pointing to an inhibition of cholinesterase or some unknown action on the acetylcholine receptors. It is unfortunate that the actions of p-benzoquinone were not examined in any of this work.

Actually one might expect acetylcholine synthesis to be more easily inhabitable than cholinesterase by the quinones; this seems to be true from the work of Torda and Wolff (1945) on frog brain, in which the synthesis is inhibited by p-benzoquinone at concentrations above 0.01 mM.
There are thus two possible actions, an increase in the sensitivity to acetylcholine and an interference with its synthesis, so that the final effect would depend on the balance of these two actions, and in addition to any direct actions on the muscle. Certainly at high quinone concentrations, where acetylcholine synthesis must be strongly inhibited, the neuromuscular failure could be attributed to this mechanism, as Torda and Wolff (1946 b) pointed out after examining \( p \)-benzohydroquinone at 9 mM. Further evidence comes from the studies on the naphthoquinones. Choline acetylase is quite potently inhibited by menadione (see Table 5-2) but cholinesterase is not affected up to 1 mM (Torda and Wolff, 1944 b). Menadione does not alter the response of frog muscle to acetylcholine, and at high concentrations (around 2.5 mM) causes contracture. Menadione at 0.01 mM increases the latency and threshold of nerve-muscle preparations, and reduces the muscle action potential magnitude, while later there is a slowing of nerve conduction and a fall in the nerve action potential (Torda and Wolff, 1949). When menadione is injected into mice and rats there is also a failure in neuromuscular transmission but little if any effect on the direct stimulation of muscle. It was believed that a depression of the synthesis of acetylcholine is the primary mechanism involved. Zefirov and Poletaev (1959) also postulated that interference with acetylcholine synthesis could explain their results with menadione at 0.57 mM. They observed a progressive neuromuscular block and a decrease in muscle contractility, accompanied by a moderate contracture, the muscle action potential duration becoming prolonged. Nerve action potentials are altered only following tetanization. Van der Kloot (1958) found that menadione-8-sulfonate above 0.1 mM causes a loss of muscle \( K^+ \) and a gain of \( Na^+ \), and thought this might be related to an inhibition of choline acetylase, but there is little evidence that this enzyme system is involved in \( Na^+ \) extrusion, and it is more likely that some inhibition of oxidative processes or an uncoupling action is responsible.

**Heart**

The heart in the whole animal seems to be relatively insensitive to the quinones and thus little attention has been given to the actions on isolated cardiac preparations. Schweitzer (1931) found, however, that the isolated frog heart is fairly susceptible to \( p \)-benzoquinone, a concentration of 0.0009 mM generally causing some effect, although concentrations of 0.009-0.09 mM are required to produce consistent and characteristic responses; higher concentrations so damage the heart that the results are difficult to interpret. He found the primary action to be on the pacemaker cells, the rate of the heart being depressed before action on the contractility is observed. This action is not mediated through vagal mechanisms since atropine does not prevent it. The contractility is eventually depressed, but may pass through a stage of mild stimulation, and the con-
duction rate is slowed. It is difficult to reconcile these results with those of Richter (1940), who reported that 0.92 mM \(p\)-benzoquinone has little effect on the cardiac rate until the contractile amplitude has been reduced 15\%, the pacemaker cells failing rather suddenly. It is possible that impurities in the quinone preparations might have accounted for some of the actions in this early work. It is interesting that 0.09–0.9 mM \(p\)-benzoquinone induces a slow development of contracture, indicating a similar effect in this regard to that on skeletal muscle (Richter, 1941).

The reports on the cardiac actions of the naphthoquinones are equally discrepant. Supniewski et al. (1936) found 1,4-naphthoquinone 0.0063–6.3 mM to progressively depress the amplitude of the frog heart, 12.6 mM causing extrasystoles, other dysrhythmias, and a semisystolic arrest. Menadione at concentrations above 0.057 depresses the amplitude but even at 11.4 mM the heart continues to beat, although at a slower rate. Phthiocol at 0.0053 mM slightly augments contractility, but at 0.053–5.3 mM there is scarcely an effect, 10.6 mM definitely depressing the contractile amplitude. Fromherz (1941) claimed that the frog heart can beat in a 1:1000 emulsion of menadione (5.7 mM) and hence is not sensitive to menadione at all. The results of Cannavà and Cavalleri (1948, 1949) are quite different, in that they found menadione to depress the frog heart above 0.011 mM and to arrest it in a few minutes at 0.28 mM. Menadiol is of similar potency and 3-methylmenadione is even more potent, so that a reaction with SH groups is made unlikely. The isolated rabbit heart is 2–4 times more sensitive than the frog heart to menadione and menadiol; the results with 3-methylmenadione may be summarized: 0.000053–0.00053 mM slightly depressant, 0.0053 mM markedly depressant with a-v dissociation, and 0.053 mM arresting. Menadione injected intravenously into dogs at 1–2 mg/kg increases the cardiac rate, depresses the t wave (which may become negative) and the st segment, elevates the p wave, and disturbs conduction, especially in the bundle. The coronary flow in rabbit hearts is increased 25\% by 0.00142 mM juglone; simultaneously there are no changes in rate or contractility, so the coronary effect must result from an action directly on the vessels (Auyong et al., 1962).

**Smooth Muscle**

The intestine of the rabbit responds to phthiocol by a progressive depression of peristalsis and tonus as the concentration is raised from 0.011 to 5.3 mM, at which complete arrest occurs (Supniewski et al., 1936). In contrast, both phthiocol and menadione injected into rabbits cause increases in motility and tonus. \(p\)-Benzoquinone arrests isolated rabbit intestine at 0.6 mM aerobically in neutral solution, but in the absence of oxygen even 1.2 mM is ineffective, and decreasing the pH abolishes the effect, presumably due to slowing the formation of the quinone (Ther
5. QUINONES
and Lüdde, 1947). However, $p$-benzoquinone at 0.6 mM acts more slowly than the hydroquinone, although the effect is superficially similar. This was explained by a slower penetration of the quinone form, whereas the hydroquinone enters rapidly but must be oxidized inside the tissue to be active. The reason for the slow penetration of the quinone was given as its tanning power, that is, its reaction with membrane proteins, presumably. These explanations are not entirely satisfactory: (1) there is no evidence in any case that the hydroquinone penetrates better than the quinone, and indeed one might predict the opposite; and (2) the role of the external pH is ignored, since presumably it would not markedly affect the oxidation of the hydroquinone inside the cells. The different intestinal muscles vary in susceptibility to $p$-benzoquinone: the longitudinal muscle is paralyzed first, following which there may be a brief period of stimulation of the circular muscle before both muscles are paralyzed and arrest occurs, no contraction being observed at any time. A study of the effects of the quinones on the responses of intestine, and other smooth muscles, to nerve stimulation and autonomic transmitters might provide interesting information.

The isolated rat uterus is stimulated by 0.053–0.53 mM phthiocol with respect to motility but the tonus is unchanged (Supniewski et al., 1936), and the guinea pig uterus is also stimulated by 0.063 mM 1,2-naphthoquinone-4-sulfonate, this being mainly with respect to the tonus (Obo, 1941 c). Blood vessels respond erratically: phthiocol contracts the intestinal vessels but dilates those in muscle, while menadione contracts both types of vessel (Supniewski et al., 1936). We noted in the previous section that juglone dilates the coronaries. Leech muscle is stimulated by 0.9 mM $p$-benzoquinone, and a similar response is given to the hydroquinone as long as the pH is neutral or higher and oxygen is present, indicating that the quinone is the active form (Labes, 1930; Bergstermann, 1944). Because of this, Bergstermann suggested that reaction with SH groups might be involved in the action, rather than participation in oxidation-reduction processes in the cells.

Responses at Sympathetic Junctions

In discussing the actions of the quinones on various tissues it is important to consider the possible interference with the formation, release, action, or metabolism of the catecholamines. The clinical use of the quinones in hypertension and how this may relate to sympathetic mechanisms will be discussed later (page 514), and here we shall confine ourselves to isolated tissue responses. Several likely mechanisms of action present themselves: (1) direct oxidation of catecholamines by quinones, (2) prolongation of the actions of catecholamines by hydroquinones by preventing their oxidation or inhibiting enzymes involved in their metabolism, (3) depression of the synthesis or release of the catecholamines, and (4) blockade of the catechol-
amine receptor sites, possibly because of a structural similarity of the quinones to the catecholamines. \textit{p}-Benzoquinone antagonizes the actions of epinephrine on the mouse uterus, rabbit intestine and heart, the ear vessels, and the toad heart, but the hydroquinone does not (Terai, 1934 a). The effects of epinephrine on the frog pupil (Terai, 1934 b) and blood pressure (Okagawa \textit{et al.}, 1934) are blocked by \textit{p}-benzoquinone, and the hydroquinone actually restores the response; this was interpreted as a direct oxidation of the ring hydroxyls of epinephrine. \textit{p}-Benzoquinone and catechol injected intravenously at a dose of 20 mg/kg in the cat produce a sensitization of the nictitating membrane to epinephrine or nerve stimulation, this being attributed to a retardation of epinephrine oxidation (Bacq, 1935). On the other hand, menadione or menadiol does not alter the blood pressure response to epinephrine (Cannavà, 1948 a). In the light of the recent advances in our knowledge of the catecholamines, a reinvestigation of the effects of the quinones might be worthwhile.

**Active Transport in Various Tissues**

Mann and Mann (1939) studied several inhibitors with the object of finding a substance, which would more or less specifically inhibit gastric acid secretion without toxic effects, for the treatment of peptic ulcer. Solutions of \textit{p}-benzoquinone were placed in the stomach or in Heidenhain pouches and the acid secretion was determined. A concentration of 0.92 mM reduces acid secretion about 50\% and is not irritating, while a concentration of 9.2 mM inhibits secretion completely for 24 hr but produces some hemorrhage. No effect was seen with 91 mM \textit{p}-benzohydroquinone or resorcinol, again demonstrating the activity of the quinone form, and possibly indicating in view of the work of Davenport on SH reagents that the action is due to the inactivation of some unknown thiol. It may also be noted that acid secretion in isolated rat stomachs is markedly depressed by 2 mM tetramethyl-\textit{p}-phenylenediamine, which may act in the quinonoid diimine form (Patterson and Stetten, 1949).

Incubation of rabbit kidney slices at 0\(^\circ\) in 0.154 \text{M} \text{NaCl} leads to an uptake of water and Na\(^+\), and a loss of K\(^+\). The presence of \textit{p}-benzoquinone at 0.5–2 mM accelerates these changes and this effect is partially reversible (Kleinzeller and Cort, 1957). One cannot say whether the action is on active transport systems or is a simple increase in permeability. The respiratory increase upon adding Na\(^+\) to dog kidney slices is not affected by \textit{p}-benzoquinone at 0.1 mM, although the normal respiration is inhibited some 12\% (Ullrich, 1958). This would be very indirect evidence that the quinone probably does not interfere with Na\(^+\) transport. Interesting results on the effects of analogs of coenzyme Q on Na\(^+\) transport have been obtained by Weinstein and Kessler (1962, 1963). The unilateral injection of Q\(_9\), 6-Br-Q\(_9\), and 6-Cl-Q\(_9\) into one renal artery leads to a unilateral natriuresis. No effect
on renal function is given by those quinones with long side chains, but
*p*-benzoquinone at a higher dosage acts similarly. All the active quinones
increase urine volume and the excretion of Na\(^+\) and K\(^+\), but have little
effect on creatinine or *p*-aminohippurate clearance. *p*-Benzohydroquinone
is not only inactive, but may reduce urine flow and Na\(^+\) excretion. It was
postulated that the Q\(_0\) analogs compete with the natural Q\(_{10}\) and thus in-
hhibit a phase of electron transport associated with Na\(^+\) resorption. Phthiocol
does not markedly effect renal processes and somewhat reduces urine flow
and Na\(^+\) excretion (Strickler and Kessler, 1963).

The Na\(^+\) transport across frog skin is inhibited by 0.01-0.1 mM *p*-benzo-
quinone although the respiration is hardly altered, and this effect seems to be
irreversible (Fuhrman, 1952). Various oxidants and reductants, in-
cluding *p*-benzoquinone, have the property of modifying Na\(^+\) transport and
the short-circuit current, but the mechanism is not clear (Eubank *et al.*, 1962).
Menadione at 0.04 mM first causes a transient increase in the short-
circuit current and trans-skin potential, but this is followed by an exponen-
tial decrease in both (Wang and Koblick, 1959). Since menadione is an
effective inhibitor of choline acetylase, it was assumed that this is the
mechanism for the depression of Na\(^+\) transport, but no direct evidence for
this site of action was presented. Taylor *et al.* (1952) propose that inhibitors
of choline acetylase and cholinesterase promote a loss of K\(^+\) from erythro-
cytes, the former by facilitating K\(^+\) efflux and the latter by inhibiting K\(^+\)
influx. Menadione at 0.1-1 mM causes a loss of erythrocyte K\(^+\) and a pro-
gressive degree of hemolysis. At 1 mM the rate of K\(^+\) is 4 times greater
than normal but cholinesterase is inhibited only 16%, while glucose utili-
ization is reduced around 50%, so it is difficult to say anything about the
site of action, especially since other changes, such as formation of methem-
globin and loss of GSH, probably also occur.

**Inflammatory Reaction**

In view of the relationship between the reaction of SH groups and in-
flammation, it is interesting to determine whether the quinones share the
irritant effects of the arsenicals, iodoacetate, and other SH reagents. The
potent bacteriostatic 3,5-dimethoxytoluquinone does not irritate when
instilled into rabbit eyes at 0.81-16 mM (Glock *et al.*, 1945), but a number
of naphthoquinones are irritant to the conjunctiva at 5%, which is so high
a concentration (250-300 mM) that the results are not very significant
(Kligman and Rosensweig, 1948). The ocular reaction to *p*-benzoquinone
was thoroughly studied by Estable (1948), who applied it in physiological
saline to rabbit eyes. Concentrations of 0.92-1.84 mM applied daily for
several days produce sensory reactions but no inflammation, whereas a
concentration of 9.2 mM produces an intense conjunctivitis, vasodilatation,
and edema, which disappear in several days. The industrial problem of
quinone damage to the eyes, arising principally from dusts in chemical plants, has attracted a good deal of attention since there is gradual development of opacity and discoloration. It is rather surprising that so little has been done on the irritant properties of the fungistatic quinones, since these will to some extent determine the clinical usefulness of these agents.

A very interesting investigation of the pain reactions and inflammation resulting from metabolic inhibition produced by the quinones was made by Herz (1954). He attempted to correlate the depression of aerobic carbohydrate metabolism with the reactions seen when the quinones are injected intracutaneously. The concentrations required to elicit pain and inflammation are shown in the accompanying tabulation; inflammation was measured by observing capillary dilatation in the frog tongue. These quinones act very rapidly and pain is usually experienced immediately upon injection. A test for inflammation in the rabbit eye to some extent parallels the vasodilatation in that 1,4-naphthoquinone is the most potent, with toluidine weaker, p-benzoquinone still weaker, and the substituted naphthoquinones relatively inactive. Insufficient enzyme or metabolic work was done to make correlations, but there seems to be a rough correlation between the abilities to produce pain and vasodilatation, although a notable exception is 3-methylenadingone, which does not inhibit oxidative enzymes well, is a weak elicitor of pain, and yet is the most potent quinone tested with respect to vasodilatation. It may well be that inhibition of keto acid oxidation is responsible for some of these actions, but additional mechanisms must be involved.

<table>
<thead>
<tr>
<th>Quinone</th>
<th>Minimal concentration for:</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Pain (mM)</td>
<td>Inflammation (mM)</td>
</tr>
<tr>
<td>1,4-Naphthoquinone</td>
<td>0.0066</td>
<td>0.02</td>
</tr>
<tr>
<td>Toluquinone</td>
<td>0.015</td>
<td>0.025</td>
</tr>
<tr>
<td>p-Benzoyquinone</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>p-Xyloquinone</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>5-Hydroxy-MD</td>
<td>0.025</td>
<td>0.005</td>
</tr>
<tr>
<td>Menadione</td>
<td>0.04</td>
<td>0.02</td>
</tr>
<tr>
<td>6-Methyl-MD</td>
<td>0.075</td>
<td>0.025</td>
</tr>
<tr>
<td>2,3-Dihydroxy-1,4-NQ</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>3-Hydroxy-MD</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>3-Methyl-MD</td>
<td>0.5</td>
<td>0.012</td>
</tr>
</tbody>
</table>
EFFECTS IN THE WHOLE ANIMAL

The pharmacological responses and the toxic patterns observed depend on the type of quinone used, the species, and the dosage, among other factors which will be considered in due course. Nevertheless, it is remarkable that rather similar toxicities have been reported for many quinones and species, and it is possible to present a general picture of the poisoning. Whether this implies a common mechanism of action is, however, debatable. The quinones have been used at one time or another in animals or man to reduce body temperature, in hypertension to reduce the blood pressure, in hypoprothrombinemic hemorrhagic conditions, in the prophylaxis and suppression of malaria, for schistosomiasis, in various bacterial and fungal infections, and as tumoristatic agents. Our purpose is to relate, whenever possible, the effects observed on the whole animal or its organs with metabolic disturbances. In the case of the quinones it is very likely that the responses to be described are the result of interactions of these quinones with enzyme systems, and yet it is difficult in a particular instance to demonstrate conclusively a selective mechanism of action, due primarily to the paucity of information available on the in vivo metabolic changes or the alterations of metabolism in the tissues themselves. However, it is hoped that, by presenting what is known of both sides of the picture, some correlations may be made and the problems for the future more clearly defined.

General Aspects of the Toxicity:

It appears that the central nervous system is the earliest and most severely affected tissue in mammals. The action is in most cases biphasic. There is usually an initial stimulation, manifested by restlessness, increased reflex excitability, muscle twitchings, ataxia, and either clonic or tonic convulsions; this may be followed by a period of depression characterized by paralysis, loss of reflex activity, respiratory depression, coma, and death. Other results of central stimulation probably are salivation, emesis, and the early respiratory irregularities and the fluctuations in blood pressure. Effects on the circulation are often not marked but there is usually a fall of the blood pressure early in the poisoning and evidences of vasodilatation. The direct effect on the heart is a slowing but initially this may be obscured by a central sympathetic discharge. Occasionally there are cardiac dysrhythmias and terminally the heart is always weak. Since isolated skeletal muscle is reasonably sensitive to the quinones, it is interesting to consider how much of the motor disturbance is central and how much peripheral. The nature of the response in most cases clearly indicates a central origin and in frogs, at least, this has been experimentally demonstrated, since the convulsions still occur following ligation of the vessels to a leg but disappear after severing the nerve. However, it is possible that peripheral effects
are exerted by higher doses and it has been noted that animals killed by
the quinones are often in a state of rigor or "stiff as boards" (Ames, 1948),
suggesting a direct production of contracture such as is seen in isolated
muscle. Several investigators have observed pulmonary edema and this
may point to an effect on the lung capillaries, particularly as petechial
hemorrhages and other signs of vascular damage have been recorded.
The renal damage reported in acute poisoning may be in part vascular but
changes in the tubular cells indicate a direct toxic effect, which would
not be surprising. Hemolysis may occur and is probably due to a direct
effect on the erythrocytes, since it occurs in vitro, and methemoglobinemia
may contribute to the lowering of the oxygen-carrying capacity of the blood.
The cause of death has been a debatable point and there have been propor-
nents for both respiratory and circulatory failure, the former being in
numerical superiority. However, the evidence for respiratory failure in
most cases has been only the observation that breathing stops almost
simultaneously with the death of the animal. In one instance it was noted
that the heart continues to beat for several minutes after cessation of the
respiration (Shimkin, 1941), but terminal ventricular fibrillation has also
been observed.

The most serious effects of the prolonged administration of the quinones
are related mainly to the blood and hematopoietic tissues. Direct effects
on the erythrocytes to favor hemolysis with the production of icterus ap-
parently unassociated with liver damage may occur, although some fatty
degeneration of both the liver and the kidney has been noted. A direct effect
on the blood-forming tissues can lead to a generalized anemia, or more spe-
cifically a granulopenia or agranulocytosis. In certain instances, particularly
with the naphthoquinones, hypoprothrombinemia and hemorrhagic states
may be induced. Animals may not gain as much weight as normally, or
may lose weight, and several investigators have stated that the animals
may die without marked symptoms but exhibiting anorexia and inanition.
There may be effects on the endocrine balance, including thyroid hyper-
plasia, and frequently either a depigmentation of the hair or production of
abnormal coloration. The pattern of chronic poisoning depends so much
on the dosage level and the species that little more of a general nature can
be said. No recent reviews on quinone toxicity are available, but the fol-
lowing general references are recommended: Ellinger (1923), Oettel (1936),
Supniewski et al. (1936), Ansbacher et al. (1942), Marquardt et al. (1947),
and Seifter (1948).

Toxic and Lethal Doses in Animals

A few representative doses are presented in Table 5-5 and from these
values some general conclusions, which may or may not prove to be valid,
can be drawn. The sensitivities of the various species are, in descending
<table>
<thead>
<tr>
<th>Quinone</th>
<th>Animal</th>
<th>Route</th>
<th>Nontoxic</th>
<th>Toxic</th>
<th>LD$_{50}$</th>
<th>LD$_{95}$</th>
<th>Reference</th>
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<td>SC</td>
<td>94</td>
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<td></td>
<td></td>
<td>Oral</td>
<td>600</td>
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<td></td>
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<td></td>
<td>Rat</td>
<td>IV</td>
<td>25</td>
<td></td>
<td>130</td>
<td></td>
<td>Woodard et al. (1949)</td>
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<tr>
<td></td>
<td></td>
<td>Oral</td>
<td>300</td>
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<td></td>
<td></td>
<td>Woodard et al. (1949)</td>
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<tr>
<td></td>
<td>Rabbit</td>
<td>IV</td>
<td></td>
<td>60</td>
<td></td>
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<td></td>
<td></td>
<td>SC</td>
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<td></td>
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<td>15</td>
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<td>p-Benzhydroquinone</td>
<td>Pigeon</td>
<td>Oral</td>
<td>300</td>
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<td></td>
<td>Mouse</td>
<td>Oral</td>
<td>400</td>
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<td>SC</td>
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<td></td>
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<td>200</td>
<td>182</td>
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<td>400</td>
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<td></td>
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<td>320</td>
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<td>Guinea pig</td>
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<td>Oral</td>
<td>400</td>
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<tr>
<td>Cat</td>
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<td>50</td>
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<td>70</td>
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<td>100</td>
<td>Oettel (1936)</td>
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<tr>
<td>Dog</td>
<td>IV</td>
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<td>88</td>
<td>Gibbs and Hare (1890)</td>
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<td>SC</td>
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<td>100</td>
<td>Brieger (1879)</td>
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**EFFECTS IN THE WHOLE ANIMAL**

*509*
<table>
<thead>
<tr>
<th>Quinone</th>
<th>Animal</th>
<th>Route</th>
<th>Dose (mg/kg)</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>2-Hydroxy-1,4-NQ</td>
<td>Mouse</td>
<td>SC</td>
<td>105</td>
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<tr>
<td></td>
<td>Guinea pig</td>
<td>SC</td>
<td>300</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Guinea pig</td>
<td>Oral</td>
<td>500</td>
<td>750</td>
</tr>
<tr>
<td>5-Hydroxy-1,4-NQ</td>
<td>Mouse</td>
<td>IP</td>
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<td>804</td>
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<tr>
<td>Menadione</td>
<td>Chick</td>
<td>Oral</td>
<td>750</td>
<td>804</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>IV</td>
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<td>130</td>
</tr>
<tr>
<td></td>
<td>Mouse</td>
<td>SC</td>
<td>18/day</td>
<td>138</td>
</tr>
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<td>IP</td>
<td>41</td>
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<tr>
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<td>Oral</td>
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<td>600</td>
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<td>Oral</td>
<td>4/day</td>
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<td>Cat</td>
<td>Oral</td>
<td>50/day</td>
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</tr>
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<td></td>
<td>Dog</td>
<td>IV</td>
<td>—</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Dog</td>
<td>IM</td>
<td>15</td>
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</tr>
<tr>
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<td>SC</td>
<td>15</td>
<td>20</td>
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<tr>
<td></td>
<td>Monkey</td>
<td>Oral</td>
<td>2/day</td>
<td>—</td>
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<tr>
<td></td>
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<td>2</td>
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<tr>
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<td>Human</td>
<td>Oral</td>
<td>1/day</td>
<td>—</td>
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<tr>
<td>Compound</td>
<td>Species</td>
<td>Route</td>
<td>Dose</td>
<td>Reference</td>
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</tr>
<tr>
<td>Menadiol</td>
<td>Mouse</td>
<td>IV</td>
<td>10</td>
<td>Shimkin (1941)</td>
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<td></td>
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<td>SC</td>
<td>36</td>
<td>Shimkin (1941)</td>
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<td></td>
<td>Oral</td>
<td>300/day</td>
<td>Ansbacher et al. (1942)</td>
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<td>IV</td>
<td>450</td>
<td>Foster (1940)</td>
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<td></td>
<td></td>
<td>SC</td>
<td>450</td>
<td>Foster (1940)</td>
</tr>
<tr>
<td></td>
<td>Rat</td>
<td>SC</td>
<td>300/day</td>
<td>Smith et al. (1943)</td>
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<td>Rabbit</td>
<td>SC</td>
<td>200/day</td>
<td>Smith et al. (1943)</td>
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<tr>
<td></td>
<td>Cat</td>
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<td>10</td>
<td>Smith et al. (1943)</td>
</tr>
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<td>Human</td>
<td>Oral</td>
<td>1.5/day</td>
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<tr>
<td>2-Methylthio-1,4-NQ</td>
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<td>SC</td>
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<td>Sakai et al. (1955)</td>
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<tr>
<td>2,3-Dichloro-1,4-NQ</td>
<td>Mouse</td>
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<td>25</td>
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<td>2-Hydroxy-3-chloro-1,4-NQ</td>
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<td>160</td>
<td>Alcalay (1947 b)</td>
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<tr>
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<tr>
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<td>Mouse</td>
<td>SC</td>
<td>105</td>
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<td>Oral</td>
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<td>Molitor and Robinson (1940)</td>
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<td>900</td>
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<td></td>
<td>Dog</td>
<td>IV</td>
<td>50</td>
<td>Wiselogle (1946 a)</td>
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<td></td>
<td>Monkey</td>
<td>Oral</td>
<td>150/day</td>
<td>Wiselogle (1946 a)</td>
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</tbody>
</table>

*a A nontoxic dose is either ineffective or exerts effects but is nontoxic; a toxic dose elicits toxic reactions but is not lethal; LD₉₅ᵢ is a dose that kills all or almost all animals. Routes are: IV = intravenous, IM = intramuscular, SC = subcutaneous, IP = intraperitoneal.*
order: cat > dog > rabbit > rat > mouse > guinea pig. For some quinones and routes the differences between the rodents are probably insignificant. Monkeys and man are undoubtedly more susceptible than the above animals but there are too few reports to evaluate this quantitatively. There is surprisingly little difference between the doses for benzo- and naphthoquinones; the mean intravenous doses for both may be given as: toxic dose 10–15 mg/kg, LD50 25–30 mg/kg, and LD95 50–60 mg/kg. The relative doses given by different routes are approximately: intravenous 1, subcutaneous 2, and oral 7.5. Such a large difference between intravenous and oral doses is to be expected in the case of substances which are unstable or easily metabolized or bound readily to the tissues. p-Benzquinone is usually 2–4 times more toxic than the hydroquinone, which is somewhat unexpected, even if the quinone form is active, since one might predict that equilibrium between the two forms would be fairly rapidly achieved in the body. The difference is seen when they are given not only by the intravenous route, but also by the subcutaneous and oral routes. That menadiol seems to be more potent than menadione is even more unexpected, but Ansbacher et al. (1942) felt this to be an artifact due to different solvents used, although the chronic subcutaneous experiments of Shimkin (1941) showed a similar relationship. Quite possibly of course the polyphenols exert some of the effects directly and do not have to be transformed into quinones; indeed, it is known that resorcinol, for example, elicits central effects similar to those produced by p-benzohydroquinone (Seifert, 1948). It is certainly true that menadiol-diP and other derivatives in which the hydroxy groups are masked are much less active. It is impossible to estimate accurately the blood concentrations produced by toxic doses, because the rates of absorption and destruction, and the fractions bound, are unknown. Yet it is quite obvious that blood levels around 1 mM could be achieved and that levels near 3–5 mM must be reached following intravenous administration. Thus the actions of the quinones on enzymes and metabolic systems at these seemingly high concentrations cannot be considered as artificial in relating them to the effects on the whole animal and attempting to ascertain the mechanism of the toxic actions. The quinones are probably not cumulative poisons generally when given daily in toxic or subtoxic doses in terms of blood or tissue concentrations, although progressive enzyme inactivation or tissue damage may occur. Sometimes near-toxic doses may be given daily without marked effects and this is undoubtedly due to the rapid metabolism of the quinones and hydroquinones in the body.

Actions on the Central Nervous System

There is good evidence that the signs of central stimulation derive from a direct action and are not the result of asphyxia, methemoglobinemia, hypoglycemia, or peripheral actions. One of the earliest actions is to induce
hyperexcitability in the spinal cord. Masing (1882) showed that the convulsions induced by \textit{p}-benzohydroquinone in frogs are actually augmented by destruction of the higher centers, although they are abolished by severing the peripheral nerves, and Labes (1929 c) obtained convulsions by injecting both the hydroquinone and the quinone into the lumbar artery of decapitated cats. Stimulation of supracord levels is indicated by restlessness, respiratory changes, emesis, various autonomic disturbances, and clonic convulsions. Higher central excitation is also seen in the fact that menadione can cause animals to come out of anesthesia (Smith \textit{et al.}, 1943). These stimulating actions have been thought to be the result of the oxidative reactions catalyzed by the quinones, but there is no evidence. There might be some insight into possible mechanisms if there were more agreement on the relative actions of the different quinones and polyphenols; but Marquardt \textit{et al.} (1947), for example, who reported interesting differences in the responses of mice to \textit{p}-benzohydroquinone, catechol, resorcinol, pyrogallol and phloroglucinol, are quite at variance with some other workers. The substances were injected subcutaneously in this study and, since many of them are locally irritating, some of the reactions may have related to the intense pain.

An interesting central effect was reported by Bijlsma and Versteegh (1922) in mice and guinea pigs following administration of \textit{p}-benzoquinone. This is a rolling motion of the body always following a rotation of the head, duplicating the response to a unilateral labyrinthine extirpation, but it was found that the labyrinth is not essential for this response and that the effect is central. Increasing dosage causes a loss of the position reflex and compensatory ocular adjustments; a cerebellar site of action is not impossible. It may be recalled that 2-phenyl-\textit{p}-benzoquinone at relatively low doses causes a peculiar writhing in mice, this being antagonized by acetylsalicylate and other analgetics (Hendershot and Forsaith, 1959).

The antipyretic activity of \textit{p}-benzohydroquinone was discovered by Brieger (1880) and its ability to reduce fever in typhoid was reported. Doses of 400–500 mg produce some cardiac weakness but no other toxic effects were noted. Steffen (1882) investigated this further and used a dose of 300–500 mg in small children and 1 g in larger children, claiming a significant antipyretic effect without toxicity. However, Oettel (1936) went over Steffen’s data and found evidence of several toxic reactions and felt that the claims were unjustified. Since these doses correspond to around 25 mg/kg, it is not surprising, as Oettel points out, that this treatment was soon discontinued. It is interesting to speculate whether these actions are similar to those of the salicylates, but they could also be due to a direct depression of metabolism.

In view of the different functions of the benzo- and naphthoquinones in electron transport, it would be important to be able to compare the central
effects accurately, but there seems to be a good deal of disagreement as to what the naphthoquinones do. Menadione and menadiol-diP injected intravenously into rabbits produce definite signs of central excitation (Foster, 1940; Fromherz, 1941), and hydrolapachol possibly exerts similar effects in dogs and man (Wiselogle, 1946 a). On the other hand, Shimkin (1941) never observed central stimulation in mice given 1,4-naphthoquinone or menadione, and Smith et al. (1943) found only evidence of depression in rats given menadiol-diP. These may be species differences but the general lack of a consistent picture of response makes impossible a discussion of the central actions of the naphthoquinones.

**Actions on the Circulation**

The quinones in toxic doses generally dilate the blood vessels, which is often obvious from the increased peripheral flow and the temperature rise in the extremities, and may damage the vessels in certain organs, such as the lungs and kidney (page 519). The effects on the blood pressure have been studied fairly thoroughly because it was thought at one time that the hypotensive activity might be clinically useful. Most of this work was done between 1942 and 1947, and was based on the demonstration by the Japanese workers in 1934 that the quinones can often block the pressor effects of the catecholamines (page 502). It was also known that menadione causes a progressive fall in the blood pressure of rabbits (Ansbacher et al., 1942). The first study of the quinones in hypertensive animals was undertaken by Friedman et al. (1942) at the Mt. Sinai Hospital, on the assumption that the quinones inactivate certain pressor amines. Rats made hypertensive by wrapping the kidneys were given various quinones subcutaneously and it was found that the blood pressure could be reduced to normal levels. When the administration is discontinued, the pressure rises again, and this cycle can be repeated several times. There is no effect on the blood pressure of normal rats at the doses used. The active compounds are p-xiloquinone, thymoquinone, trimethyl-p-benzoquinone, and sodium rhodizonate. The effect is not immediate and requires 3–5 days to reach a maximum. It was shown later (Oppenheimer et al., 1944) that similar effects are exerted by 1,4-naphthoquinone, menadione, and 1,2-naphthoquinone-4-sulfonate, whereas juglone and 3-methylmenadione are inactive. However, the toxic effects of these naphthoquinones were considered to limit their clinical usefulness. Injections of 5–10 mg menadione into hypertensive rats cause a fall in the blood pressure which is maximal around the 4th day, and after the injections are stopped the pressure rises again (Schwarz and Ziegler, 1944).

It was thought at this time that the ischemic kidney releases pressor amines which might be inactivated by the quinones. The failure to exert a hypotensive action in normotensive rats at the doses employed seems to
indicate that the sympathetic catecholamines are not primarily involved. Although quinones can oxidatively deaminate certain amines in vitro, it is very unlikely that this reaction occurs in vivo, and indeed there is evidence that the quinones can interfere with the normal enzymic metabolism of amines. Beyer (1946), on the basis of unpublished clinical results by others, and the failure to find an antihypertensive action in species other than the rat — e.g., Goldblatt could observe no blood pressure lowering in hypertensive dogs — concluded that the hypotensive action in rats is a special case and is probably not related to the destruction of amines. This pronouncement seems to have terminated study in this field and the clinical value of such quinones has not been adequately determined, nor has the mechanism of the hypotensive action been elucidated. Although it would be very optimistic to believe that the ordinary quinones might be of clinical value in hypertension, it might be worthwhile to investigate the effects of the quinones on formation, release, action, and metabolism of angiotensin.

Actions on the Blood and Hematopoietic System

The in vivo formation of methemoglobin has been observed in dogs given p-benzoquinone (Gibbs and Hare, 1890), catechol (Heubner, 1913), menadione, and menadiol (Cannavà, 1948 a, b); in rabbits given p-benzoquinone (Ellinger, 1923) and menadione (Fromherz, 1941); in rats given p-benzoquinone (Ellinger, 1923); and in cats given p-benzohydroquinone (Jung and Witt, 1947). The amount of methemoglobin present in the blood is never very high, even following toxic doses of the quinones, and is usually only in trace quantities. The levels may occasionally fall after an initial rise. Thus it seems quite clear that neither the acute nor chronic toxic actions of the quinones are due to the elevation of methemoglobin levels, although combined with other actions leading to a reduction in erythrocytes and hemoglobin it might contribute slightly. The formation of methemoglobin in vitro when blood is incubated with p-benzoquinone was demonstrated by Heubner (1913). No methemoglobin is produced by p-benzohydroquinone anaerobically, but upon admission of oxygen the sequence Hb → HbO₂ → met-Hb was observed. That this is a simple direct oxidation of hemoglobin is indicated by the fact that the reaction occurs when crystalline horse hemoglobin is mixed with p-benzoquinone. Nevertheless, Heubner found very little methemoglobin formed in dogs injected with p-benzohydroquinone or catechol. Oettel (1936) claimed that the lack of a marked in vivo action is due to the fact that the blood contains substances maintaining the hydroquinone in a reduced form and, in a series of experiments involving acute and chronic administration of p-benzoquinone to several species, found little or no methemoglobin in the blood, even at the time of death, although methemoglobin formed more
readily post-mortem in the blood of poisoned animals. The formation of methemoglobin in vitro by the quinones is catalytic, as shown by the very low minimal concentrations necessary — durohydroquinone (0.00028 mM), p-benzoquinone (0.061 mM), menadione (0.00063 mM), 3-methylmenadione (0.0054 mM), and 2-ethyl-1,4-naphthoquinone (0.00054 mM) — the quinones being reduced by various reactions in the erythrocytes (Fromherz, 1941). The relation of the formation of methemoglobin to the effects of the quinones on erythrocyte respiration have been discussed previously (page 489). The rates at which methemoglobin is formed in dog blood incubated with various quinones were determined by Hoffmann-Ostenhof et al. (1947 a), who felt there to be no relationship to redox potential or the inhibition of any enzymes (see accompanying tabulation). The

<table>
<thead>
<tr>
<th>Quinone</th>
<th>Methemoglobin formation (mg/100 ml/hr)</th>
<th>$E'_o$ (pH 7) (v)</th>
</tr>
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<tr>
<td>2,6-Dichloro-p-Q</td>
<td>107</td>
<td>+ 0.31 (?)</td>
</tr>
<tr>
<td>1,2-Naphthoquinone</td>
<td>64</td>
<td>+ 0.127</td>
</tr>
<tr>
<td>p-Benzoquinone</td>
<td>50</td>
<td>+ 0.284</td>
</tr>
<tr>
<td>Methylphthazarin</td>
<td>33</td>
<td>— 0.15 (?)</td>
</tr>
<tr>
<td>Toluquinone</td>
<td>29</td>
<td>+ 0.24</td>
</tr>
<tr>
<td>1,4-Naphthoquinone</td>
<td>22</td>
<td>+ 0.07</td>
</tr>
<tr>
<td>Phthazarin</td>
<td>20</td>
<td>— 0.11 (?)</td>
</tr>
<tr>
<td>5-Methoxy-TQ</td>
<td>17</td>
<td>+ 0.13</td>
</tr>
<tr>
<td>2,5-Dimethoxy-p-Q</td>
<td>15</td>
<td>+ 0.06</td>
</tr>
<tr>
<td>2,6-Dimethoxy-p-Q</td>
<td>12</td>
<td>+ 0.11 (?)</td>
</tr>
<tr>
<td>Lawson</td>
<td>8</td>
<td>— 0.139</td>
</tr>
</tbody>
</table>

lack of a general correlation with redox potential may be due to various factors, but it would be difficult to evaluate these without information on the relative rates of the direct oxidation of hemoglobin. In any event, one must assume that hemoglobin, along with numerous other substances in the blood, is able to reduce quinones and hence possibly alter their toxicity, as Liu (1928) suggested.

The chronic administration of p-benzoquinone, menadione, and phthiocol brings about rather marked falls in erythrocytes and hemoglobin, agranulocytosis, and often pancytopenia (Kracke and Parker, 1934; Molitor and Robinson, 1940; Ansbacher et al., 1942; Jung and Witt, 1947). Since the quinones are antimitotic one might expect a depression of the hematopoietic tissues. In most instances it requires 2–4 weeks for hematopoietic depression to be manifest and it often occurs with quite small doses, e. g., 15 mg/day of p-benzoquinone injected into rabbits. Some of the erythrocyte and hemoglobin reduction may be the result of hemolysis; indeed, where this
is marked at least a temporary stimulation of the hematopoietic system has been noted. The hemolytic action of the quinones has been mentioned frequently (Oettel, 1936), but whether this is the result of a direct action on the erythrocytes or to be attributed to some action on the liver has been debated. In favor of the former hypothesis, quinones have been shown to be hemolytic in vitro, and structural changes in the circulating erythrocytes have been observed during menadione administration (Fromhertz, 1941). Menadione is hemolytic at concentrations between 0.00063 and 0.0063 mJ. However, 1,2- and 1,4-naphthoquinone are much less active on human blood, hemolysis being produced only by concentrations above 1 mJ (Rieders and Brieger, 1951). No in vivo hemolysis was observed with these substances. There is little if any direct evidence in favor of the liver hypothesis; although liver damage has been reported following toxic doses of the quinones, there is no reason to relate this to hemolysis, and indeed hemolysis and anemia will occur at doses much lower than those giving rise to significant liver changes.

Inasmuch as vitamin K is a naphthoquinone required for the synthesis of prothrombin in the liver, there is the possibility that certain naphthoquinone derivatives might depress prothrombin synthesis by competitive interference and hence lead to a hemorrhagic state. Woolley (1945 a) tested 2,3-dichloro-1,4-naphthoquinone in weanling mice on a vitamin K-deficient diet and found that it reduces the survival time. However, the animals do not bleed and the prothrombin is normal, and furthermore neither vitamin K nor menadione protects. It appears that this derivative is incapable of interfering with prothrombin synthesis. On the other hand, 2-chloro-1,4-naphthoquinone and 3-hydroxy-2-chloro-1,4-naphthoquinone are weak antagonists of vitamin K and can produce a deficiency state characterized by prolongation of the clotting time (Meunier et al., 1945). It may be mentioned that some of the antimalarial 2-hydroxy-3-alkyl-1,4-naphthoquinones produce a hemorrhagic syndrome without damaging the liver or reacting with prothrombin directly, and the administration of vitamin K₁ is effective in preventing this (C. C. Smith et al., 1946).

Factors Altering the Susceptibility to Quinones

Vollmer and his colleagues at Breslau studied the effects of various factors on the toxicity of p-benzohydroquinone in order to determine if there is a relationship to the metabolic rate of the animals. His basic concept was that the toxicity of a hydroquinone is dependent on its oxidation to the corresponding quinone, and that the more active the metabolism of the animal, the more readily would this occur and the greater the toxicity. Administration of thyroxine to mice until they are hyperthyroid increases the susceptibility to p-benzohydroquinone; at a dose of 150 mg/kg subcutaneously, the normal mice mainly recover whereas the hyperthyroid
mice die (Vollmer and Buchholz, 1930). The administration of lactate for several days also increases the susceptibility to the hydroquinone. Vollmer (1932 a) pointed out that the toxicity depends on the weight of the animal; at a dose of 200 mg/kg subcutaneously, $p$-benzohydroquinone kills over 90% of mice under 14 g, but less than 45% of mice over 16 g. This was related to the higher rate of metabolism in the smaller animals. Mice made tolerant to alcohol are more sensitive to $p$-benzohydroquinone, and again this was interpreted in terms of metabolic levels, the alcohol-resistant animals being thought to have a faster metabolism (Vollmer, 1932 b). Obviously, such results are open to other interpretations and it is well known, for example, that age and weight influence the susceptibilities to many drugs where metabolic rate is probably not the factor involved. It is difficult to see how lactate could increase the oxidizing ability in the blood or tissues, and the hyperthyroid mice might suffer from metabolic disturbance more readily than normal mice because of the greater energy requirements of their actively functioning tissues. Marquardt et al. (1947) repeated the work on thyroxine and examined several phenols and polyphenols (see accompanying tabulation). A ratio less than 1 indicates that hyperthyroidism reduces the susceptibility. Vollmer's results with $p$-benzohydroquinone were not confirmed, and indeed there was a small decrease in susceptibility. The effect of thyroid seems to depend on the nature of the substance tested and probably on the mechanisms involved in the toxicity.

**Poisoning in Man**

Five cases of severe poisoning by $p$-benzohydroquinone have been found in the literature. These have resulted mainly from the ingestion of photo-

<table>
<thead>
<tr>
<th>Quinone or quinol</th>
<th>$LD_{50}$ (mg/kg)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Hyperthyroid</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>213</td>
<td>286</td>
</tr>
<tr>
<td>Catechol</td>
<td>108</td>
<td>140</td>
</tr>
<tr>
<td>$p$-Benzoquinone</td>
<td>87.6</td>
<td>93.8</td>
</tr>
<tr>
<td>$p$-Benzohydroquinone</td>
<td>182</td>
<td>193</td>
</tr>
<tr>
<td>Phloroglucinol</td>
<td>991</td>
<td>1050</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>566</td>
<td>490</td>
</tr>
<tr>
<td>Phenol</td>
<td>359</td>
<td>286</td>
</tr>
<tr>
<td>Hydroxy-$p$-QH$_2$</td>
<td>371</td>
<td>229</td>
</tr>
</tbody>
</table>
graphic developer, either accidentally or with suicidal intent. In some instances the effects of \( p \)-benzohydroquinone may be modified by catechol present, but in any case one is dealing with the effects of hydroquinones. The earliest report was by Rémont and Colombies (1927) on a salesman who had taken 12 g hydroquinone. He first experienced a buzzing in the ears, constriction in the throat, and respiratory difficulty, and was soon unable to make voluntary movements. He was in a state of marked prostration, with slowly developing cyanosis, the respiration being labored and rapid. The pulse was rapid but regular, and the blood pressure and reflexes were normal. The blood picture was not abnormal except for hypoglycemia and the urine was black. He began to recover and by the fourth day the urine was of normal color. It is interesting that central stimulation, so notable in most laboratory animals, was not observed, and that there was a lack of hepatic, renal, or hematological toxicity.

Halbron et al. (1931) reported the case of a Negress who took 5 g of \( p \)-benzohydroquinone and 4.5 g of catechol. During the first day she lost consciousness and had convulsions, but by the second day consciousness returned. The urine was black but there was no albumin or methemoglobin. After the fourth day there was icterus, accompanied by a fall in the erythrocyte count. She died on the twelfth day and autopsy showed only hemorrhagic petechiae in the gastrointestinal tract, with hemorrhagic infiltration of the liver and biliary pigmentation of the parenchymal cells. Here it would seem that the primary toxic actions were exerted initially on the central nervous system and later on the blood and liver. Busatto (1939) reported a female who took 6 g \( p \)-benzohydroquinone and 2 g \( p \)-methylaminophenol sulfate. During the following 4 days there was hypotension, a weak pulse, cyanosis, and some icterus. The icterus then increased markedly and hyperthermia developed. She died on the sixth day and autopsy showed biliary pigmentation, pulmonary edema, bronchopneumonia, a cloudy swelling of the kidney, fatty degeneration of the liver, and acute myocarditis. Quite possibly all of these were not due to the substances ingested. Again there was no mention of central stimulation. Zeidman and Deutl (1945) discussed two cases in which approximately 15 g of developer had been taken, with the primary result a hemolytic anemia with jaundice, the toxic effects being directed here on the blood and liver. Except for the relative lack of central effects, these poisonings are reasonably in accord with those reported in animals. Carlson and Brewer (1953) stated that the ingestion of 300–500 mg \( p \)-benzohydroquinone daily for 3–5 months by 10 human subjects produced no changes in the blood or urine, indicating that this substance is not very toxic and that its effects are not cumulative.
**ANTIMITOTIC ACTIONS**

Despite the fact that the quinones have long been known to inhibit the growth of various microorganisms, invertebrates, and plants, direct demonstration of a true antimitotic action was first made by Lehmann in 1942. Since that time the principal streams of work may be designated as follows: Lehmann and his collaborators at Bern from 1942 to 1949 using *Tubifex* eggs; Meier and his collaborators at Basel from 1945 to 1947 using tissue cultures; the Cambridge school of Mitchell, Friedmann, Simon-Reuss, Hughes, and others from 1947 to 1956 using fibroblasts and tumor cells; Parmentier and Dustin at Brussels from 1948 to 1953 on *in vivo* antimitotic effects; and Druckrey and his colleagues at Freiburg from 1952 to 1954 using sea urchin eggs. By *antimitotic action* is meant at least a partially selective inhibition or blockade of some step in the mitotic cycle without simultaneously killing the cells or obviously damaging them. Many quinones undoubtedly have an antimitotic effect in this sense, and this selective toxicity on dividing cells has stimulated interest in the quinones as of possible clinical value in the suppression of tumors. Thus the most comprehensive, quantitative, and interesting data we have on the quinones relate to their antimitotic activity. We shall see that the basis for this activity has been postulated to be an action on metabolic systems, but the mechanisms involved are debatable.

**Inhibition of Egg Cleavage**

Lehmann introduced the eggs of the fresh-water oligochaete *Tubifex* as an experimental object in 1942, and in succeeding reports (Lehmann, 1945, 1947, 1949; Lehmann and Hadorn, 1946; Lehmann and Bretscher, 1951) has shown their cleavage to be very sensitive to many quinones, the effect being selective in that no gross nuclear damage can be observed. This action was analyzed and compared with the actions of other antimitotic agents in a thorough manner, and we shall attempt to present the salient features of this work.

The effects exerted by the quinones can usually be classified into three categories depending on the concentration and the total time of exposure. In the lowest concentration range, cleavage is not inhibited but development is disturbed so that abnormal blastulas are formed; at somewhat higher concentrations, cleavage itself is blocked; and finally, at still higher concentrations, damage to the cells occurs as shown by cytolysis. It is true that cytolysis does not occur at very high concentrations, the eggs being presumably killed in a fixed or gel state, but this is far above the antimitotic range in which we are interested. These changes are plotted in Fig. 5-6 for *p*-benzoquinone; initially there is a rapid drop in normal embryos, with a simultaneous rise in defective blastulas; it is only after this
effect has reached its peak that somewhat higher concentrations begin to block cleavage. It may also be noted that cytolysis begins to occur at the same concentration as mitotic blockade, so that \( p \)-benzoquinone is not a specific antimitotic at any concentration. However, it is possible by treatment of the eggs for short periods to induce mitotic blockade without subsequent cytolysis. The effects of several quinones are shown in Fig. 5-7 and their potencies and types of action are compared with colchicine and diethylstilbestrol. It is interesting that \( p \)-benzoquinone is at least 100 times more potent than colchicine and 15 times more potent than diethylstilbestrol, but does not possess the specificity of action evident with these drugs, neither of which seems to induce cytolysis even at high concentrations. This implies that the cytolysis might be characteristic of the action of quinones, and it is observed with other quinones (except for 9,10-anthraquinone, which is not antimitotic, or 5,6-benzanthraquinone, where solubility limits the testing at higher concentrations). The lack of obvious correlation between structure and either potency or type of action is remarkable. It may be questioned as to how much of the effect is due to the quinone groups and how much to the polycyclic ring systems, especially as polycyclic aromatic compounds are capable of exerting potent effects on cleavage and development. However, there is certainly no general increase in potency with increase in the number of rings, \( p \)-benzoquinone being at least as po-

![Fig. 5-6. Effects of \( p \)-Q on the development of *Tubifex* eggs. (A) Normal embryos; (B) abnormal blastulas; (C) cytolized cells; (D) mitotically blocked cells. (From Lehmann and Hadorn, 1946.)](image-url)
tent as some naphtho- and anthraquinones. In fact, 9,10-anthraquinone is not antimitotic, whereas 1,2-anthraquinone is quite potent, indicating that neither the quinone structure nor a polycyclic system is sufficient for activity. It is also remarkable that the addition of a ring onto 1,2-anthraquinone to form 5,6-benzanthraquinone increases the antimitotic

<table>
<thead>
<tr>
<th>Quinone</th>
<th>Concentration (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colchicine (0.083)</td>
<td></td>
</tr>
<tr>
<td>Diethylstilbestrol (0.0074)</td>
<td></td>
</tr>
<tr>
<td>1,2-Anthraquinone (0.00048)</td>
<td></td>
</tr>
<tr>
<td>9,10-Anthraquinone</td>
<td></td>
</tr>
<tr>
<td>1,4-Naphthoquinone (0.00063)</td>
<td></td>
</tr>
<tr>
<td>p-Benzquinone (0.00046)</td>
<td></td>
</tr>
<tr>
<td>9,10-Phenantrahydroquinone (0.0002)</td>
<td></td>
</tr>
<tr>
<td>5,6-Benzanthraquinone (0.000013)</td>
<td>Limit of solubility</td>
</tr>
</tbody>
</table>

*Fig. 5-7. Effects of various quinones on the cleavage of Tubifex eggs. The ranges of effects are indicated as follows: diagonally lined area — development disturbed; clear area — mitosis inhibited; and black areas — cytolysis produced. The figures give the minimal antimitotic concentrations (mM). (From Lehmann, 1947.)*
potency 30- to 40-fold and induces a specificity not seen in the former compound. It is unfortunate that, as in most work of this type, there are no data available on nonquinonoid polycyclic compounds, although it is known certainly that phenanthrene and benzanthracene exert fairly potent antimitotic actions on other cells.

Lehmann divided antimitotics into two general categories on the basis of their cytoplasmic and nuclear actions:

(I) Inhibitory antimitotics: inhibit cytoplasmic movement, and hence cleavage, without significantly affecting the nucleus (e.g., p-benzoquinone and 1,4-naphthoquinone).

(II) Destructive antimitotics: interfere with and destroy the nuclear apparatus (karyolytic action), without affecting cytoplasmic movements, so that cells may divide with defective nuclei (e.g., colchicine, diethylstilbestrol, and 9,10-phenanthraquinone).

Such differences in action introduce further complexities and make it doubtful that substances appearing to exert similar effects in Fig. 5-7 really act by identical mechanisms. It may be mentioned that other benzoquinones studied (toluquinone, methoxytoluquinone, and chloranil) all seem to act like p-benzoquinone in having little effect on the nucleus. The substituted naphthoquinones (e.g., menadione) also probably behave similarly to the parent compound, whereas 1,2-naphthoquinone only cytolyzes and is not directly antimitotic. If one might hazard a guess, the polycyclic structure may be effective in disturbing nuclear function and modifying embryonic development, whereas an active quinone tends to cause damage to the plasma membrane, leading to cytolysis; since cleavage can be stopped by a number of mechanisms, possibly either structure interferes. It seems very unlikely that a compound could exert a specific effect on cytoplasmic movements without simultaneously depressing certain other cell functions and producing damage, the degree depending on the duration of exposure. The nature of the nuclear action is unknown, but it was postulated that the blocking of cleavage by p-benzoquinone is due to the alteration of cytoplasmic structure from a sol to a gel state, a reaction involving structural proteins rather than enzymes. However, in view of the recent work demonstrating a relationship between the sol-gel state, metabolism, and ATP, an effect on enzymes cannot be excluded.

It was shown that p-benzoquinone, when present in low concentration, is taken up rapidly and completely by eggs, whereas colchicine is taken up slowly and only in unmeasurable quantities. Using small volumes of fluid, Lehmann calculated the amount of quinone taken up by the eggs (see accompanying tabulation). He termed this a “biological titration” of the quinone, since one must specify the absolute amounts required to produce specified effects. Nevertheless, it must be remembered that not necessarily
all of the quinone taken up is involved in the actions observed, and that a large fraction may be combined with cell components of no immediate consequence in mitosis. It establishes only an upper limit to the amount required.

<table>
<thead>
<tr>
<th>Amount (g/egg)</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>$6 \times 10^{-10}$</td>
<td>None</td>
</tr>
<tr>
<td>$15 \times 10^{-10}$</td>
<td>Abnormal mitosis and development</td>
</tr>
<tr>
<td>$20-25 \times 10^{-10}$</td>
<td>Inhibition of cleavage</td>
</tr>
<tr>
<td>$45 \times 10^{-10}$</td>
<td>Cytolysis</td>
</tr>
</tbody>
</table>

It would be highly desirable to have more thorough studies on the metabolic alterations, if any, induced by the antimitotic concentrations of these quinones. It is true that none of the enzymes tested is as sensitive to $p$-benzoquinone as is cleavage in these eggs, but most of the important enzymes have not been examined. The role of the plasma membrane in cleavage has been largely ignored in most studies; it is entirely possible that effects on permeability, membrane potentials, or organizing patterns could be of importance, and work on membrane properties would also be highly desirable before mechanistic interpretations are made.

A comparison of the antimitotic and antidevelopmental potencies of several quinones on the eggs and embryos of Tubifex and Paracentrotus is shown in Table 5-6. The activities against each type of egg are roughly parallel. The loss of potency upon methylation, especially of 1,4-naphthoquinone, is notable.

Further correlations between structure and activity are provided in the work of Druckrey and his colleagues (Druckrey et al., 1952, 1953) on the eggs of Paracentrotus lividus (see accompanying tabulation). Phenolic

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration (mM) for 50% inhibition of cleavage</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p$-Benzoquinone</td>
<td>0.0074</td>
</tr>
<tr>
<td>Phenol</td>
<td>3.2</td>
</tr>
<tr>
<td>$p$-Benzohydroquinone</td>
<td>0.0045</td>
</tr>
<tr>
<td>Catechol</td>
<td>0.14</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>&gt;2.7</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>0.8</td>
</tr>
<tr>
<td>1,4-Naphthoquinone</td>
<td>0.05</td>
</tr>
<tr>
<td>1,4-Naphthohydroquinone</td>
<td>0.013</td>
</tr>
<tr>
<td>1,2-NQ-4-sulfonate</td>
<td>0.8</td>
</tr>
<tr>
<td>$\alpha$-Naphthol</td>
<td>0.14</td>
</tr>
<tr>
<td>$\beta$-Naphthol</td>
<td>0.10</td>
</tr>
<tr>
<td>Table 5-6</td>
<td>Effects of Quinones and Colchicine on Cleavage and Development</td>
</tr>
<tr>
<td>-----------</td>
<td>------------------------------------------------------------</td>
</tr>
<tr>
<td>Substance</td>
<td>Concentrations (mM)</td>
</tr>
<tr>
<td>p-Benzoquinone</td>
<td>0.0046</td>
</tr>
<tr>
<td>Toluquinone</td>
<td>0.0041</td>
</tr>
<tr>
<td>1,4-Naphthoquinone</td>
<td>0.00063</td>
</tr>
<tr>
<td>Menadione</td>
<td>0.011</td>
</tr>
<tr>
<td>9,10-Phenanthraquinone</td>
<td>0.00066</td>
</tr>
<tr>
<td>5,6-Benzanthraquinone</td>
<td>0.00097</td>
</tr>
<tr>
<td>Colchicine</td>
<td>0.31</td>
</tr>
</tbody>
</table>

*From Lehmann and Bretscher (1951).*
groups per se are not sufficient for inhibitory activity and o-diphenols are weak; only the \textit{para} compounds are highly active and this led Druckrey to postulate a "\textit{para} principle," which may be merely an expression of the need for a structure which can react readily with SH groups or possesses bifunctionality for cross-linking. The hydroquinones are somewhat more potent than the corresponding quinones in the two instances tested and it was stated that the oxidation of the hydroquinones is presumably not necessary for activity, a conclusion not completely justified because of possible differences in permeability of the cells to the two forms. Another factor may be the effect of the oxidation of the hydroquinones on the eggs, since Runnström (1932) showed that sea urchin eggs can utilize hydroquinone as a carrier and that it is oxidized through a cyanide-sensitive system, probably cytochrome oxidase. It may well be that in some cases one cannot attribute activity solely to either the quinone or hydroquinone, since the mechanism may involve a redox couple in which both forms are equally important.

\textbf{Disturbances in Embryonic Development}

Several groups of substances are known which might be said to possess two or more reactive groups and which interfere with mitosis, cause fragmentation and cross-linking of chromosomes, and in general duplicate the effects of irradiation. Druckrey (1952) used \textit{p}-benzoquinone as a model substance of bifunctional type and tested it on the development of free-swimming blastulas of \textit{Paracentrotus lividus}. A concentration of 0.01 mM causes a sticking together of the blastulas after several hours, conglomerates of several blastulas after 1 day, and frequent giant blastulas of spherical form after 3 days; these remain motile and capable of development. After 9 days there are monsters composed of two or more embryos, and in some cases giant plutei are formed. Earlier stages are more sensitive, because egg cleavage would have been quite depressed by this concentration. It appears, however, that such effects may be unrelated to nuclear, chromosomal, or mutagenic activity, and are conceivably mediated through actions on the membranes or cytoplasm of the cells; the results do not provide much evidence for the validity of the polyfunctional group hypothesis.

Abnormalities in morphogenesis are produced by 0.22–0.44 mM menadiol-diP in frog and chick embryos (Bellairs, 1954). Mitotic effects are observed within 2 hr and a certain degree of cytoplasmic vacuolation is evident. The most frequent abnormality is a failure of the neural folds to close in the region of the presumptive hindbrain, due, it is suggested, to interference with mitoses in the neural plate. Other abnormalities are failure of the two sides of the foregut floor and the two heart vesicles to unite ventrally, reduction in blood island formation, and decrease in the embryonic size.
Inhibition of Mitosis in Tissue Cultures

The numerous reports of the Cambridge group represent perhaps the most thorough investigation of any aspect of quinone action, and the application of these findings to cancer therapy has begun (see page 541). On the basis of the work of Lehmann on Tubifex eggs, Mitchell in 1946 began a study of the effects of menadione on chick fibroblast mitosis. Initially he used menadiol-diP because it is water-soluble and felt the action might be more selective if the active groups were blocked and released later within the cells. Exposure of the cultures to menadiol-diP for 24 hr leads to inhibition of mitosis (see accompanying tabulation) (Mitchell and Simon-Reuss, 1947). However, it was soon found (Friedmann et al., 1948a) that 1,4-naphtohydroquinone-diP is much more potent, being approximately 1000 times more active than the methyl derivative, and has an effective threshold concentration of 0.0000005 mM. Mitosis is almost completely blocked at 0.0005 mM. No toxic effects are seen at 0.000001 mM but at 0.00001 mM there are some vacuolized and exploded cells. Extensive comparisons of the actions of various quinones were made later and some of the results are summarized in Table 5-7. Before considering the relationships between structure and activity, it will be necessary to discuss some of the cytological changes induced by these agents since there are some qualitative differences in their actions.

Mitotic inhibition by the quinones may or may not be associated with disturbances in the phase distribution, abnormal mitoses (clumping of chromosomes at metaphase, anaphase bridges, and chromosomal fragmentation and deletions), and toxicity to the cells (cytoplasmic bubbling, enlargement, and rounding up), and quite certainly more than a single mechanism of action is involved. Menadiol-diP, for example, first causes temporary cell enlargement, this occurring within 2 min at 0.005 mM, and cytoplasmic bubbling accompanied by swelling of mitochondria and nuclei (Mitchell and Simon-Reuss, 1952 b). These changes reach a maximum at 20 min and then disappear slowly. Mitotic inhibition is obvious after 80 min and persists for 24-36 hr, the commonest abnormality being a clumping

<table>
<thead>
<tr>
<th>Concentration (mM)</th>
<th>% Inhibition of mitosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0025</td>
<td>18</td>
</tr>
<tr>
<td>0.003</td>
<td>31</td>
</tr>
<tr>
<td>0.004</td>
<td>48</td>
</tr>
<tr>
<td>0.005</td>
<td>77</td>
</tr>
<tr>
<td>0.006</td>
<td>76</td>
</tr>
</tbody>
</table>
### Table 5-7

**Antimitotic Potencies of Quinones and Polyphenols in Fibroblast Cultures**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Concentration (mM) for 50% mitotic inhibition</th>
<th>Reference&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p$-QH$_2$-diP</td>
<td>0.000003</td>
<td>(5)</td>
</tr>
<tr>
<td>$p$-QH$_2$</td>
<td>0.000006</td>
<td>(5)</td>
</tr>
<tr>
<td>Resorcinol</td>
<td>0.00001</td>
<td>(2)</td>
</tr>
<tr>
<td>Resorcinol-diP</td>
<td>0.0005</td>
<td>(2)</td>
</tr>
<tr>
<td>Phenol-P</td>
<td>0.0005</td>
<td>(2)</td>
</tr>
<tr>
<td>Phenol</td>
<td>0.001</td>
<td>(2)</td>
</tr>
<tr>
<td>Trimethyl-$p$-QH$_2$-diP</td>
<td>0.005</td>
<td>(5)</td>
</tr>
<tr>
<td>$\alpha$-Cresol</td>
<td>0.008</td>
<td>(2)</td>
</tr>
<tr>
<td>TQH$_2$-diP</td>
<td>0.01</td>
<td>(5)</td>
</tr>
<tr>
<td>TQH$_2$</td>
<td>0.05</td>
<td>(5)</td>
</tr>
<tr>
<td>$\alpha$-QH$_2$ (catechol)</td>
<td>Inactive</td>
<td>(2)</td>
</tr>
<tr>
<td>Tetramercaptoacetic-$p$-Q</td>
<td>Stimulates</td>
<td>(3)</td>
</tr>
<tr>
<td>1,4-NQH$_2$-diP</td>
<td>0.000003</td>
<td>(5)</td>
</tr>
<tr>
<td>1,4-NQH$_2$</td>
<td>0.000005</td>
<td>(2)</td>
</tr>
<tr>
<td>2-Hydroxy-3-mercaptopoacetic-1,4-NQ</td>
<td>(0.00004)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(3)</td>
</tr>
<tr>
<td>2,3-Dimercaptoacetic-1,4-NQ</td>
<td>(0.0003)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>(3)</td>
</tr>
<tr>
<td>MDH$_2$-diP</td>
<td>0.0038</td>
<td>(4)</td>
</tr>
<tr>
<td>Phthiocol</td>
<td>0.004</td>
<td>(5)</td>
</tr>
<tr>
<td>2-Bromo-1,4-NQ</td>
<td>0.004</td>
<td>(1)</td>
</tr>
<tr>
<td>2-Bromo-1,4-NQH$_2$-diP</td>
<td>0.004</td>
<td>(5)</td>
</tr>
<tr>
<td>2-Carboxyl-1,4-NQH$_2$-diP</td>
<td>0.005</td>
<td>(5)</td>
</tr>
<tr>
<td>3-Bromo-MDH</td>
<td>0.006</td>
<td>(1)</td>
</tr>
<tr>
<td>Juglone</td>
<td>0.006</td>
<td>(3)</td>
</tr>
<tr>
<td>3-Bromo-MDH$_2$-diP</td>
<td>0.007</td>
<td>(5)</td>
</tr>
<tr>
<td>3-Methyl-MDH$_2$-diP</td>
<td>0.007</td>
<td>(5)</td>
</tr>
<tr>
<td>2,3-Dicarboxyl-1,4-NQH$_2$-diP</td>
<td>0.009</td>
<td>(5)</td>
</tr>
<tr>
<td>Lawsone</td>
<td>0.01</td>
<td>(3)</td>
</tr>
<tr>
<td>MD</td>
<td>0.02</td>
<td>(5)</td>
</tr>
<tr>
<td>Vitamin K$_1$</td>
<td>Inactive</td>
<td>(5)</td>
</tr>
</tbody>
</table>

---

<sup>a</sup> The references are: (1) Friedmann et al. (1952 a); (2) Friedmann et al. (1954); (3) Friedmann and Simon-Reuss (1956 a); (4) Mitchell and Simon-Reuss (1947); and (5) Mitchell and Simon-Reuss (1952 b).

<sup>b</sup> Estimated.
of the chromosomes with some fragmentation, but no anaphase bridges are evident. Mitoses can be inhibited without marked mitotic abnormalities at the proper concentration. Some compounds, such as 2-bromo-1,4-naphthohydroquinone-diP, 2,3-dicarboxyl-1,4-naphthohydroquinone-diP, or trimethyl-p-QH₂-diP, are quite toxic to resting cells whereas other compounds show a much more selective action on cells in mitosis. Some compounds seem to alter phase distribution markedly so that, in one case, there is an accumulation of metaphase cells; others do not alter this pattern and instead prevent entrance into mitosis. As in eggs, some compounds act more on cytoplasmic and others on nuclear processes. The three principal types of action with respect to fibroblast division are: (1) blockade of entry into mitosis, (2) metaphase arrest with spindle abnormalities, and (3) chromosomal breakage and clumping. An interesting point, as yet unsettled, is the possible role of cell edema in causing the alterations in spindle and chromosomes structure and behavior. That is, do the quinones act directly on the mitotic apparatus or indirectly affect it through disturbances in cell permeability, active transport, and water balance? It may perhaps be important to remember that 2,4-dinitrophenol at 0.005 mM produces cell swelling, clumped metaphases, and chromosomal fragmentation, so that an interference with the energy metabolism of the fibroblasts can bring about many of the changes seen with the quinones.

It is difficult to discuss structure-action relations in view of the different types of action, and the following general remarks must be taken as being only tentative.

(A) Phosphorylation. The phosphorylated compounds are usually 2-5 times more potent antimitotically than the corresponding hydroquinones. The reason for this is unknown. If the phosphates are hydrolyzed in the cell by phosphatases to the active compounds, one would not expect the phosphates to be more potent unless penetration into the cells is facilitated. There are no data on relative penetration rates, but one would expect the phosphates to enter the cells less readily, due to the introduction of negative charges onto the molecules. The question as to whether the phosphates can act directly cannot be answered because no work has been done with isolated systems and under conditions in which the phosphates are assuredly stable. The suggestion by Mitchell and Simon-Reuss (1952 b) that the di-phosphates may react with polypeptide chains, particualrly with the basic amino acids such as arginine in chromosomal histones, because of a corresponding phosphate-to-phosphate distance of around 10 Å, is interesting but requires confirmation. If this occurs, it might interfere with the union of nucleic acids to proteins. But if the phosphates are indeed active, then how do the nonphosphorylated compounds act to produce similar antimitotic effects?

(B) Methylation. Methylation of both benzo- and naphthoquinones re-
ducès the activity, sometimes quite markedly. It is interesting to compare the compounds in the following tabulation:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (mM) for 50% mitotic inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) 1,4-NQH₂-diP</td>
<td>0.000003</td>
</tr>
<tr>
<td>(II) Menadion-diP</td>
<td>0.0038</td>
</tr>
<tr>
<td>(III) 3-Methylmenadion-diP</td>
<td>0.007</td>
</tr>
</tbody>
</table>

Although (I) is much more potent than (II), it does not exhibit such selectivity; (II) produces few mitotic abnormalities and does not alter the phase distribution, whereas (I) causes many abnormalities and metaphase accumulation. Compound (III) differs from the others in that it blocks the entrance of the cells into mitosis at higher concentrations, but at very low, not antimitotic, concentrations it causes metaphase accumulation, which increases with the concentration. Compound (III) brings about spindle abnormalities such as are induced by colchicine, and causes marked chromosome fragmentation and anaphasic bridging. It was postulated that the two methyl groups sterically hinder the free rotation of the phosphate groups, imposing a distance between them comparable to the repeating distance along a polypeptide chain. These three substances were compared on cultures of chick osteoblasts by Hughes and Simon-Reuss (1953). Here the basic action of the three is metaphase arrest, due primarily to a failure in the formation of the spindle, or its disorientation if already formed, the chromosomes clumping secondarily. There is no effect on the entry into prophase. All cause cellular edema. After exposure to (I) at 0.000001 mM for 6 hr there are a number of binucleate cells, indicating a primary effect on cytoplasmic cleavage mechanisms. That the penetration of such substances into cells is possibly quite slow was pointed out, since some actions appear only after many hours, but it is more likely that such lag periods are dependent on delay in the cellular response.

(C) Bromination. Introduction of a bromine atom in the 2-position of 1,4-naphthoquinone reduces the antimitotic activity much as does a methyl group. MDH₂-diP and 2-Br-1,4-NQH₂-diP have essentially the same potency, but the latter is more toxic and induces metaphase accumulation in a higher concentration range. 3-Methyl-MDH₂-diP and 3-Br-MDH₂-diP also are equally potent but the patterns of action are somewhat different. As Mitchell and Simon-Reuss (1952 b) pointed out, the methyl and bromine groups have about the same radii but the inductive effects are different, bromine being more electronegative, so that the differences in action may be related to alterations in the rest of the naphthoquinone structure (e. g., as manifested by different redox potentials or reactivities with thiols).
(D) Hydroxylation. Both lawsone and juglone are presumably less potent than 1,4-naphthoquinone (this quinone was not tested but is probably of comparable potency to the corresponding hydroquinone), but the addition of a hydroxy group to menadione to form phthiocol somewhat increases the potency, although it weakens the ability to produce metaphase arrest, an effect attributed by Mitchell and Simon-Reuss (1952b) to the electron-repelling inductive property of the hydroxy group. 2,3-Dihydroxy-1,4-naphthoquinone produces metaphase block but has lost the capacity for inhibiting the entry into prophase.

(E) Carboxylation. The introduction of carboxyl groups reduces the potency somewhat more than do methyl or bromo groups, but the differences are not as much as might be expected from the increased negative charge.* The 2,3-dicarboxyl-1,4-\(\text{NQH}_2\)-diP type of action resembles that of the dimethyl derivative, although metaphase effects and spindle abnormalities are not so common, indicating either the relative unimportance of these groups in the over-all action, or the oxidation of methyl to carboxyl groups in the cell.

(F) Cyclic versus acyclic compounds. In discussing structure-action relations, one must also consider the early hypothesis of Friedmann et al. (1948a) that perhaps only the \(-\text{CO}—\text{CH}=\text{CH}—\text{CO}—\) grouping is responsible for the antimitotic effects, since maleate and some related compounds exert effects similar to those discussed above. Maleate at 0.002 mM inhibits mitoses almost 70% and alters the phase distribution (see page 322), with clumped metaphases, undivided telophases, and chromosomal fragmentation. The concentration for 50% mitotic inhibition is given as 0.0005 mM, so that it is certainly of comparable potency with the quinones. If this hypothesis is valid, it implies that the quinonoid structure is not necessary, the active grouping being the conjugated double-bond system which reacts with SH or other groups in the cell. This problem will be taken up in more detail in the discussion of the possible mechanisms involved (page 533).

Further information on the actions of menadiol-diP may be found in the work of Richard et al. (1954) on fibroblast cultures from embryonic mouse heart. These cells seem to be at least 10 times more sensitive than chick fibroblasts, but the important additional fact emerging is the critical

* The effects of phosphorylation and carboxylation of the hydroquinones lead to the question whether these compounds must get into the cells or whether they can exert their antimitotic actions on the cell membrane. On the other hand, possibly fibroblasts are not so impermeable to anions as are most cells, or possess mechanisms for the uptake of such substances, either intact or metabolically altered. The surface motility evident in time-lapse studies of fibroblasts in culture may indicate that pinocytosis occurs and accounts for the uptake of these anions.
role of the exposure duration. With any concentration used, 50-min exposure is required if 100% mitotic inhibition is to be achieved. At a concentration of 0.00033 mM, the inhibition increases from 56% to 100% as the exposure time is increased from 30 to 60 min. Even concentrations as low as 0.0000033 mM definitely inhibit if allowed to act for 60 min. If the cells are exposed to 0.00055 mM menadiol-diP for 30 min and then washed, the antimitotic action is evident later, indicating that the inhibitor is either bound tightly or produces some irreversible modification.

It may also be noted that Meier and Allgöwer (1945) claimed that $p$-benzoquinone is a typical premitotic inhibitor, in contrast to colchicine which interferes with cleavage, and that at low concentrations near 0.000009 mM the antimitotic action is selective, no toxic effects on fibroblasts being observed (Meier and Schär, 1947). Although fibroblasts are very sensitive to $p$-benzoquinone, lymphocyte cultures from rat lymph nodes are not, 50% inhibition being seen at 0.19 mM (Trowell, 1960). A general review of the effects of quinones on mitosis has been given by Biesele (1958).

We shall now turn our attention to the suggested mechanisms by which the quinones inhibit mitosis, and especially to the evidence for and against the participation of SH groups in the critical reaction, a theory which dominated the early work of the Cambridge group. The major difficulty in accepting this is that the antimitotic activities do not parallel the reactivities with SH groups. One may argue that penetration or other factors are involved, but the fair degree of activity of the 2,3-disubstituted 1,4-naphthoquinones (such as 3-methyl-MDH$_2$-diP and phthiocol), which do not react with SH groups, cannot be explained on this basis. Also, if the phosphates are directly active, and we have seen there is some evidence for this, the SH reaction theory would not be applicable. The greater potencies of the unsubstituted benzo- and naphthohydroquinones might be attributed to a reaction with SH groups in addition to whatever mechanisms are involved in the actions of the substituted compounds. On the other hand, some substances which react rather readily with thiols, such as 2-bromo-1,4-naphthoquinone or menadione, are not particularly potent antimitotic agents.

Another approach to determining the role of the thiols in the actions of the quinones is the examination of various conjugates of the quinones with the thiols. Some of these conjugates have been shown to be quite potently antimitotic and the results are very puzzling. The compound formed by the addition of glutathione to menadione, for example, is more active than menadione (Mitchell and Simon-Reuss, 1952 b). On the other hand, the addition of mercaptoacetate to menadione yields an inactive product, and the addition of both glutathione and mercaptoacetate to 1,4-naphthoquinone also gives inactive compounds (i.e., no mitotic inhibition or phase shifts at concentrations up to 0.006 mM). This work was extended by Friedmann and Simon-Reuss (1956 a) and the results are
summarized in Table 5-8. Compounds III and IV are at least 100 times more potent than the others and one must concede the marked structural requirements and the inactivating effects of certain groups; e. g., the losses of activity when a 2-methyl group is substituted for a 2-hydroxy group, or methyl groups are put on the mercaptoacetic side chains, or these side chains are slightly lengthened. Compounds III and IV cause metaphase accumulation at low concentrations and simultaneously produce cell edema and occasionally cytolysis. Compound VII actually accelerates mitosis, while it has no effect on the phase distribution and does not produce mitotic abnormalities. It is interesting to note that the addition of the mercaptoacetic group to lawsone to give Compound III increases the potency well over 100-fold.

One must consider several possibilities in attempting to interpret the results of such studies. (1) The compounds are unstable within the cell and split off the mercaptoacetic group to form the active parent quinone. This explanation is very unlikely for several reasons: as far as is known these groups are not easily split off, it is difficult to understand why Compound III is more potent than lawsone, and it is not clear why Compound I is not the most active since removal of the mercaptoacetic group would produce the active 1,4-naphthoquinone. (2) The compounds such as III and IV are directly active. If this is so, one might propose that the quinones enter the cells and form toxic conjugates with soluble thiols, an interesting possibility for which there is no experimental evidence. (3) These compounds may be metabolized to toxic products within the cells. It is known that the mercaptoacetic derivatives are easily oxidized to sulfoxides (Friedmann and Simon-Reuss, 1956 b). The conjugates with mercaptoacetate and other thiols are thioethers, and the actions of certain thioethers (e. g. ethionine) are antagonized by other thioethers (e. g. methionine). It was found that the mitotic inhibition produced by Compound IV could be effectively counteracted by methionine. Methionine forms an energy-rich complex with adenosine (S-adenylmethionine) and it was suggested that the sul-

\[
\text{Sulfoxide of Compound III} \quad \text{S-Adenylmethionine}
\]

foxides of Compounds III and IV might compete with this onium complex, possibly to interfere with methyl transfers. If this were true, it would be an entirely new type of action unassociated with the quinone structure, and would explain the actions of the quinones only if thiol conjugates
### Table 5-8

**Effects of Addition Compounds of Quinones and Thiols on Fibroblast Mitosis**

<table>
<thead>
<tr>
<th>Number</th>
<th>Compound</th>
<th>Concentration (mM)</th>
<th>% Mitotic inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td><img src="image" alt="Compound I" /></td>
<td>0.01</td>
<td>72</td>
</tr>
<tr>
<td>II</td>
<td><img src="image" alt="Compound II" /></td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td><img src="image" alt="Compound III" /></td>
<td>0.00005</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.0001</td>
<td>87</td>
</tr>
<tr>
<td>IV</td>
<td><img src="image" alt="Compound IV" /></td>
<td>0.0001</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.001</td>
<td>73</td>
</tr>
<tr>
<td>V</td>
<td><img src="image" alt="Compound V" /></td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td>VI</td>
<td><img src="image" alt="Compound VI" /></td>
<td>0.01</td>
<td>0</td>
</tr>
<tr>
<td>VII</td>
<td><img src="image" alt="Compound VII" /></td>
<td>0.001</td>
<td>Stim 43</td>
</tr>
</tbody>
</table>

*From Friedmann and Simon-Reuss (1956).*
were formed in the cells. It would be important to know if such reactions apply to the conjugates of the quinones with cysteine or glutathione. This theory does not very well explain the relative inactivities of Compounds I, II, and VI.

Relatively little attention has been given to the possibility that the hydrogen peroxide formed during the oxidation of the hydroquinones is at least partly responsible for the mitotic inhibition. Dold et al. (1963) pointed out that hydrogen peroxide is formed particularly in glycolyzing cells such as the Ehrlich ascites carcinoma and felt that it is a primary factor in the effects of 9,10-phenanthrahydroquinone, while Kayser (1964) suggested that autoxidation of this substance produces enough hydrogen peroxide to account for the effects, and showed that 0.0025 mM 9,10-phenanthrahydroquinone depresses glycolysis 92% if the incubation is aerobic but has no effect under anaerobic conditions. Catalase is also able to protect the cells.

Radiosensitizing Action

Certain quinones potentiate the antimitotic action of X-irradiation on fibroblasts and for this reason have been termed "radiosensitizers" (Mitchell and Simon-Reuss, 1947, 1952 a). This action could well be an important one in the clinical use of the quinones in tumor therapy. Menadiol-diP has been especially studied in this respect and the results shown in the accompanying tabulation are typical. The inhibition with the combined

<table>
<thead>
<tr>
<th>Treatment</th>
<th>(\text{Menadiol-diP} ) (mM)</th>
<th>(\text{X-irradiation} ) (r)</th>
<th>% Mitotic inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.002</td>
<td>—</td>
<td>150</td>
<td>30.1</td>
</tr>
<tr>
<td>0.002</td>
<td>—</td>
<td>150</td>
<td>23.2</td>
</tr>
<tr>
<td>0.004</td>
<td>—</td>
<td>150</td>
<td>83.8</td>
</tr>
<tr>
<td>0.004</td>
<td>—</td>
<td>300</td>
<td>45.2</td>
</tr>
<tr>
<td>0.004</td>
<td>—</td>
<td>300</td>
<td>42.2</td>
</tr>
<tr>
<td>0.004</td>
<td>—</td>
<td>300</td>
<td>94.1</td>
</tr>
</tbody>
</table>

* The % inhibitions are not to be summed even though the actions are additive. In the first experiment the predicted inhibition from combined treatment would be around 46% and in the second experiment around 68%, so that the experimental results give inhibitions 36–38% greater than expected.
types of inhibition are different, which is substantiated by the quite different cytological sequences observed. Marrian (1959) postulated that X-irradiation interferes with DNA synthesis whereas menadiol-diP inhibits the synthesis of RNA.

Mitotic Inhibition in Vivo

Parmentier and Dustin investigated the antimitotic effects of quinones and hydroquinones on the germinal zone of the mouse intestine. Such an effect had first been observed by Zylberszac (1939) following injections of \( p \)-benzohydroquinone, and Dustin (1947) had reported extensive nuclear degeneration occurring before the onset of prophase 4 hr after the injection of 125 mg/kg of \( p \)-benzohydroquinone. Parmentier and Dustin (1948) believed that a new type of mitotic abnormality is produced. During the first 2 hr there is a progressive accumulation of arrested metaphases, and these generally show small groups of chromosomes migrating toward the poles, although most of the chromosomal material remains at the equatorial plate — these were later termed “three-group metaphases” (Parmentier, 1953 a). The central mass is not composed of fragments of broken chromosomes, nor are they excessively clumped at this stage (although they clump later), nor is there evidence of spindle fiber detachment. They favored the view that division of centromeres is suppressed, especially in the longer chromosomes. Catechol also exerts this action. It was supposed that oxidation to the quinone forms occurs and is followed by reaction with SH groups (Parmentier, 1949). The general pycnencrosis produced is similar to that seen after X-irradiation. Mitotic abnormalities in the ovary, uterus, intestine, and some epithelia are produced in the rabbit by \( p \)-benzohydroquinone at doses of 15–175 mg/kg subcutaneously or intraperitoneally (Parmentier, 1953 b). Rats respond similarly, while bone marrow changes can be demonstrated in the hamster (Parmentier, 1953 a). However, Parmentier's explanation was now different. The chromatic material near the poles was believed to have been left there pre-metaphase, rather than migrating there from the equatorial plate. The question then is: Why does this material not migrate to the equator properly when the bulk of the chromosomes does? In human bone marrow cells, \( p \)-benzohydroquinone has a similar effect and the smaller chromosomes do not migrate to the plate; it was observed that the chromatic substance is altered, assuming a fluidlike state until it coagulates into spherical masses. This work was extended by Rosin and Doljanski (1953) to chick fibroblasts, and the liver, cornea, and bone marrow of the rat \textit{in vivo}. Exposure to \( p \)-benzohydroquinone at 0.009 mM for 1 hr gives typical three-group metaphase cells in fibroblasts and this \textit{in vitro} effect at least indicates that the action is direct and not through secondary factors. It is rather remarkable that such definite nuclear changes can be induced in animals by nonlethal doses of
Antimitotic Actions in Plant Cells

*p*-Benzoquinone has been found to inhibit the germination of seeds and the growth of seedlings and roots, but very different degrees of potency have been reported. For example, wheat seedlings are inhibit and injured by 0.0092 mM *p*-benzoquinone (Schreiner and Reed, 1907), but the germination of cress seeds is not affected at 1 mM, although it is blocked completely at 10 mM (Schmid, 1944). Much of the variation is probably due to the experimental differences in the treatment of the seeds, the durations of exposure, and other factors. Hoffmann-Ostenhof and Reitmaier (1948) compared the effects of various quinones on the germination and growth of cress seeds, and generally found the naphthoquinones to be more potent inhibitors than the benzoquinones, both 1,2- and 1,4-naphthoquinone depressing growth around 50% at 0.1 mM. *p*-Xyloquinone is only a little less active, while *p*-benzoquinone is one of the weakest quinones, toluidine being the least active of all. The proliferation of blue-green algae is suppressed very potently by 2,3-dichloro-1,4-naphthoquinone, the most active of all the inhibitors examined, 0.00009 mM being effective (Fitzgerald et al., 1952). It is difficult to interpret studies of over-all growth depression, because several types of mechanism may be imagined, and one cannot attribute the results to an antimitotic action without direct evidence.

Mitotic effects have been reported several times and the findings may help to throw some light on the problem of antimitotic mechanisms. Nybom and Knutson (1947) observed an unusual type of mitotic abnormality in onion root cells treated with menadione; the chromosomes divide into two or more groups at the poles during anaphase, and this was called “distributed c-mitosis” (c-mitosis designates a colchicine-like effect). This was more extensively studied by Levan and Tjio (1948), who showed that *p*-benzoquinone, *p*-benzohydroquinone, catechol, *p*-phenylenediamine, and related compounds are all active. Two types of chromosomal fragmentation occur: free fragments left at the equator at anaphase, and attached fragments that retain a vestige of connection to the parent chromosomes. Translocations are quite rare and reattachment occurs in only a few instances. In addition, it was thought that impeded division of the centromeres of the longest chromosomes contributes to the abnormal mitosis. *p*-Benzohydroquinone causes c-mitosis in concentrations from 5 to 100 mM, so that these cells are not nearly as sensitive as mammalian cells. *p*-Benzoquinone is active at about one tenth these concentrations and some fragmentation occurs at 0.02 mM. Loveless and Revell (1949) could detect no chromosome breakage in *Viciaa* roots treated with *p*-benzohydroquinone and felt that the quinones are not radiomimetic, a conclusion supported
by the fibroblast work discussed above. Yakar (1952) found c-mitoses in onion and tiger lily root tips treated with 1 mM p-benzohydroquinone, the spindles inactivated, and the contracted chromosomes scattered throughout the cytoplasm in closely appressed c-pairs, chromosomal breakage occurring after 24 hr.

An interesting idea was expressed by Reed (1949) with regard to the possible effects of the naturally occurring quinones on plant growth. Plants with certain mineral deficiencies are dwarfed and poorly differentiated; simultaneously an excess of quinones appears within the cells, due perhaps to a shift in the intracellular redox potential. It was postulated that these quinones might block mitosis and retard growth and differentiation. A study of zinc-deficient walnut trees was done; the leaves here are curled and dwarfed, and contain coacervated phenolic material and active polyphenol oxidase. Some of the palisade cells are blocked in mitosis and become hypertrophied. He then tested the effect of phenanthraquinone at 0.00048 mM on the meristematic cells of Tradescantia buds and showed chromosomal distortion and mitotic abnormalities. Some relation was claimed to exist between these quinone effects and the changes seen in the walnut leaves. The relation is indeed tenuous, but it is an intriguing approach to morphological disturbances due to deficient nutrients in plants. Actually the phenolic compounds or quinones occurring naturally in the walnut leaves should be extracted and tested directly, since their actions might be quite different from phenanthraquinone (even the effects of juglone would be more informative).

After presenting these investigations on antimitotic activity, one is impressed by the need to determine more closely, if possible, the site or sites of action for the induction of these mitotic inhibitions. At present some workers are inclined to believe in a direct chromosomal action, others in effects on the spindle, others in secondary effects subsequent to cell edema due to actions on membrane mechanisms, and others to inhibition of metabolic systems involved in mitotic movements. We lack studies on (1) the metabolic changes occurring in cells undergoing disturbances in mitosis, (2) the changes in SH groups or thiols within cells acted upon by the quinones, (3) the exact chronological sequence of the effects in cells, and (4) a quantitative comparison of these actions with those of other SH reagents in order to determine more accurately how much the reaction with these groups contributes to the mitotic interference.

**EFFECTS ON NEOPLASTIC CELLS**

As is the case with many substances which may inhibit neoplastic growth, the quinones seem to be able under certain circumstances to induce tumors, and we shall briefly consider this aspect before the inhibitory properties
are taken up. Takizawa in a series of reports in 1940–1941 (see Hartwell, 1951) claimed that the application of \( p \)-benzoquinone, 1,4-naphthoquinone, and phenanthraquinone in benzene at concentrations usually of 5–12 mM to the skin of mice often results in papillomas, a certain fraction of these becoming malignant as epitheliomas. The appearance of these tumors is preceded by depilation and increased keratinization, with some local necrosis of the epithelium (Takizawa, 1940). Chloro-\( p \)-benzoquinone, thymoquinone, and 1,2-naphthoquinone are inactive. \( p \)-Benzoquinone and chloranil given daily for 40 days subcutaneously or intravenously to rabbits do not produce tumors (Hartwell, 1951). Gwynn and Salaman (1953) found that 1,4-naphthoquinone and menadione cause epidermal hyperplasia when applied at 33 mM in acetone to mouse skin, but 1,2-naphthoquinone and \( p \)-benzoquinone do not. 9,10-Dimethyl-1,2-benzanthracene, which alone causes benign papillomas, was applied simultaneously, and none of the quinones altered the frequency of tumor induction. Tiedemann (1953) painted mouse skin with 92 mM \( p \)-benzoquinone in benzene and with benzene alone; in both cases there is depilation, epidermal necrosis, inflammation, and the production of papillomas, but no carcinomas, thus casting some doubt on the carcinogenicity of \( p \)-benzoquinone. It is still undecided if such quinones can produce tumors, but in any event they must be of rather low potency. The inhalation of \( p \)-benzoquinone vapor for 1 hr 6 times a week by mice leads to an 8% incidence of lung carcinoma (Kanisawa and Ide, 1959), but whether this is a specific or nonspecific effect is unknown.

It has been suggested that certain carcinogens are oxidized to the active forms (Potter, 1942; Kensler et al., 1942 a, b), these products possibly reacting with and inhibiting SH enzymes and NAD-linked systems. \( p \)-Dimethylaminobenzene is carcinogenic and its oxidative products are quite potent inhibitors of succinate oxidase and pyruvate decarboxylase, and this was discussed with regard to the shifting of metabolism from aerobic to anaerobic dominance. One factor determining which enzymes are attacked within the cells would be the degree of saturation of the enzymes with substrate or coenzyme; thus it was postulated that 3-phosphoglyceraldehyde dehydrogenase may not be as readily inhibited as succinate oxidase because it was assumed to be saturated with substrate under most conditions. Although this can be a factor modifying the rate of enzyme inhibition, there is a question as to its importance, since some inhibitors, such as iodoacetate, seem to attack the 3-phosphoglyceraldehyde dehydrogenase preferentially. It has also been suggested at times that \( p \)-benzoquinone may be the actual carcinogenic substance produced from a variety of carcinogens, but there is no evidence for this at present.

Several reports have demonstrated some selective inhibition of tumor growth by various quinones administered to tumor-bearing animals. Sub-
stituted quinones injected intraperitoneally twice daily in Twort carcinoma-bearing mice for approximately 2 weeks cause an inhibition of the tumor growth (see accompanying tabulation) (Powell, 1944). The animals gain weight during this period, indicating a specific effect on the tumor tissue. The feeding of 9,10-PAQ at 1-2% in the diet to mice inhibits the growth of Carcinoma 63, and a fibrosarcoma is inhibited even more; indeed, occasional complete regression was observed (Powell, 1951). Adenocarcinomas, squamous cell carcinomas, sarcomas, and a spindle-cell tumor are also inhibited. Powell observed some reaction of these substances with proteins and believed that reaction with SH groups might be important. It is very difficult, as previously discussed, to be certain that the activity is associated with the quinone structure when the polycyclic compounds are used, since nonquinonoid analogs or similarly substituted saturated hydrocarbons are not used as controls. The uptake of these quinones by tumor tissue is well shown in the work of Lewis and Goland (1947), who found that after 16 days the tumors in mice fed 0.25% 1,4-diamino-9,10-AQ are reduced to one fourth the original size and are stained purple. Sakai et al. (1955) found that intraperitoneal injections of 10-20 mg/kg/day of 2-methylthio-1,4-NQ or 2,3-dimethylthio-1,4-NQ prolong the lives of mice inoculated with Ehrlich ascites carcinoma cells, and felt that the methylthio derivatives give the best promise for suppression of tumors in vivo. Fukuoka et al. (1957) suggested that these carsinostatic quinones suppress tumor growth through an inhibition of glycolysis because of a close correlation of the two activities. On the other hand, quinones have been found to stimulate tumor growth. Badger et al. (1942) observed an increase in the size of implanted Walker rat carcinoma 256 during the administration of 1,4-naphthoquinone and menadione. It is interesting to note that duroquinone at rather high doses had no effect on the tumor growth; there has been very little study of the benzoquinones in this connection.

The most intensive investigation of this subject has been made by the group at Cambridge in relation to the general problem of the antimitotic

<table>
<thead>
<tr>
<th>Quinone</th>
<th>Dose (mg)</th>
<th>Interval (days)</th>
<th>% Tumor inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-Dihydroxy-5,8-NQ</td>
<td>0.125</td>
<td>14</td>
<td>90.6</td>
</tr>
<tr>
<td>1,2,4-Trihydroxy-9,10-AQ</td>
<td>0.125</td>
<td>14</td>
<td>55.0</td>
</tr>
<tr>
<td>1,2,4,5,6,8-Hexahydroxy-9,10-AQ</td>
<td>0.05</td>
<td>13</td>
<td>46.9</td>
</tr>
<tr>
<td></td>
<td>0.125</td>
<td>14</td>
<td>81.3</td>
</tr>
<tr>
<td>9,10-PAQ</td>
<td>0.05</td>
<td>8</td>
<td>52.1</td>
</tr>
</tbody>
</table>
action, this work continuing from 1947 to the present time, Mitchell (1948) first used menadiol-diP (Synkayvite), on the basis of its potent antimitotic and radiosensitizing actions on fibroblasts, in the treatment of clinical neoplasms. In 116 patients with advanced malignant tumors it produced in many cases a definite palliative improvement in conjunction with X-irradiation; in bronchial carcinoma the life-span was increased significantly and there was a palliative response in 23 of 73 other types of tumor. The usual dose was 100 mg either intramuscularly or intravenously, the latter being better, along with radiation therapy. Toxic effects were minimal, there being some transient nausea and focal pain due to cellular edema in the tumor. It was shown later that sections of the tumors from treated patients exhibit areas of gross necrosis, blocked mitosis, and pycnolic nuclear degeneration with some chromosomal fragmentation (Mitchell, 1949). The menadiol-diP treatment of patients with other than bronchial carcinomas followed for 3.5 years was summarized by J. S. Mitchell (1953), who stated, “The results suggest that the proportion of cases showing unexpectedly good clinical response is greater with radiotherapy combined with the compound administered by intravenous injection than with radiotherapy combined with the compound administered by intramuscular injection.” He believed it to have a small but useful effect as a radiosensitizer. The mean survival of bronchial carcinoma cases was raised from 5.9 to 10.8 months when intravenous injections of menadiol-diP were used. It has been claimed that menadiol-diP-2-C^* given to Walker carcinoma-bearing rats is concentrated more in the tumor than in other tissues, but analysis of the data shows that, although this is true for muscle, it is not generally true* (Marrian and Maxwell, 1956). We may also mention the recent report of Deeley (1962) confirming the results of Mitchell, menadiol-diP prolonging survival of patients with bronchial carcinoma over that of patients receiving radiation alone.

The report by Friedmann and Bailey (1950) on the treatment of rats with Jensen sarcoma by intramuscular injections of 1,4-naphthoquinone-diP and menadiol-diP, in which it was claimed that little or no effect was observed (although tumor weight fell from 22.48 to 18.73 g), cannot be seriously considered as a true test of the compounds since only a single dose by a rather unfavorable route was used. Mitchell’s work has been criticized by Gellhorn and Gagliano (1950) on the basis of a lack of proper criteria for a beneficial palliative effect, and they could find no suppressive effect of menadiol-diP on various transplanted tumors in animals; however, the substance was given intraperitoneally, which is probably

* Many substances, particularly those with ionic groups, do not enter skeletal muscle as readily as they do most other tissues, as has been clearly shown in much recent work with drugs, so that a tumor-muscle ratio greater than unity for menadiol-diP does not signify a special concentrating mechanism.
not an effective route at the dosage used.* Jolles and Laws (1954) thought the improvement in patients given menadiol-diP might be due to an increased tolerance to the X-irradiation, but their work with animals did not support this view, no constant effect being observed.

The mechanism by which quinones interfere with tumor cell mitosis and growth is probably the same as that involved in the inhibition of normal cells, such as fibroblasts, but some workers have assumed qualitative differences. Powell (1944, 1951) thought that cancer cells have a modified cytoskeletal structure, fibrils associated with mitosis being disarticulated. Substances such as the quinones which link together these fibrils and thus stabilize the malignant cells in a more normal arrangement were believed to slow division. However, such a hypothesis has no experimental basis, none of his work had any bearing on this concept, and the substances chosen as antimitotic agents (e.g., 9,10-AQ and 9,10-PAQ) certainly seem to be unlikely candidates for fibril-linkers (in fact, it is doubtful if they react readily with SH groups). Menadione or menadiol-diP has no cross-linking ability, and we have seen that Mitchell, Friedmann, and others have provided evidence that if reaction with SH groups is involved at all, it must be the low molecular weight thiols rather than the protein components of the cells. It is tempting to entertain the hypothesis in view of the great potency of some of these compounds that a very specific structure or process is attacked, this being solely associated with mitosis. The inactivation of some component of the centriole, for example, might produce a failure in spindle function and chromosomal movements. In any case, there is no evidence for a qualitatively different action on tumor cells, nor is there reason to believe that quinones selectively suppress neoplastic growth. It is possible, as Holzer (1956) has suggested, that the high glycolytic activity of tumor cells makes them more susceptible than normal cells to substances which depress glycolysis, and it is true that certain carcinostatic agents potently inhibit glycolysis, but there is certainly no reason to believe that the antimitotic quinones affect glycolysis at all in concentrations blocking cell division.

* There has been much discussion, and rightly so, during the past few years of the usual lack of critical evaluation in the clinical testing of drugs, but few seem to have realized that one must examine reports of negative results just as sceptically as those of favorable results. There are several instances in which obviously active drugs have been proved to be ineffective by inadequate techniques. If an investigation is designed to demonstrate the incorrectness of someone's previous work or to prove the inadequacy of some therapeutic procedure, some attempt should be made to reproduce the previous work with respect to doses and routes of administration, which was not done in the case cited. It must furthermore be remembered that experiments with a single dose and route of administration provide information only on the effectiveness under these conditions, even though the work was done very thoroughly and evaluated by adequate criteria.
The potent antibacterial and antifungal activities of certain quinones have led to their trial for use as antiseptics and in the treatment of systemic infections. It is possible that some plants or their extracts used by primitive peoples or in folk medicine owe their activity to naturally occurring quinones. The leaves and berries of many of the Ericaceae have been widely used for centuries in the treatment of urinary tract infections. *Arctostaphylos uva-ursi* (bearberry) is the best known of these and contains appreciable amounts of arbutin (\( p \)-benzohydroquinone-\( \beta \)-D-glycopyranoside) in the leaves, the ingestion of which leads to the appearance of \( p \)-benzoquinone in the urine (Madaus and Koch, 1939). The golden rhododendron has been used medicinally in Siberia and contains 4% arbutin and 0.76% \( p \)-benzohydroquinone in the leaves, so that extracts inhibit the growth of several pathogens (Medvedeva, 1952). In the search for effective antibiotics occurring in fungi and plants it was soon recognized that many of the active substances are quinones (Florey *et al*., 1949; Huisman, 1950), and it was postulated that the antibacterial action might be related to the reaction with SH groups (Geiger, 1946; Rinderknecht *et al*., 1947). Some simple antibiotic quinones are fumigatin from *Aspergillus fumigatus*, spinulosin from *Penicillium spinulosum*, and javanicin from *Fusarium javanicum*. Other antibiotics, such as geodin and penicillic acid, contain the grouping

\[
\text{CH}_3 \quad \text{C} = \text{C} - \text{C} = \text{O}
\]

but are not true quinones. The antibacterial activity of these and other quinones has stimulated much screening work to find compounds useful in agriculture and medicine, but there has been very little investigation of the metabolic effects or the mechanisms of inhibition. Some of the results of this work are summarized in Table 5-9, from which it is clear that various quinones completely prevent the growth of gram-positive bacteria at concentrations in the range 0.01-0.05 mM.

The results in Table 5-9 are not to be considered as quantitatively
### Table 5-9

**Inhibition of Bacterial Growth by Quinones**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inhibitor</th>
<th>Bacteriostatic concentration (mM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gram-positive bacteria</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus mycoides</em></td>
<td>TQ</td>
<td>0.032</td>
<td>Kavanagh (1947)</td>
</tr>
<tr>
<td></td>
<td>MD</td>
<td>0.068</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3,5-diCH$_3$O-TQ</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>MD</td>
<td>0.006</td>
<td>Geiger (1946)</td>
</tr>
<tr>
<td></td>
<td>2,6-diCH$_3$O-p-Q</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TQH$_2$</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Juglone</td>
<td>0.013</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-CH$_3$-6-CH$_3$O-p-Q</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2,6-diCH$_3$p-Q</td>
<td>0.018</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2,6-diCl-p-Q</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TQ</td>
<td>0.027</td>
<td></td>
</tr>
<tr>
<td></td>
<td>p-QH$_3$</td>
<td>0.030</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DQ</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>TriCl-p-Q</td>
<td>0.039</td>
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</tr>
<tr>
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<td>2,3-diCl-1,4-NQ</td>
<td>0.044</td>
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</tr>
<tr>
<td></td>
<td>p-Q</td>
<td>0.046</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,4-NQ</td>
<td>0.052</td>
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</tr>
<tr>
<td></td>
<td>TriCH$_3$p-Q</td>
<td>0.056</td>
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</tr>
<tr>
<td></td>
<td>Phthiocoll</td>
<td>0.133</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Chloranil</td>
<td>0.41</td>
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</tr>
<tr>
<td></td>
<td>TQ</td>
<td>0.008</td>
<td>Kavanagh (1947)</td>
</tr>
<tr>
<td></td>
<td>MD</td>
<td>0.017</td>
<td></td>
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<td></td>
<td>3,5-diCH$_3$O-TQ</td>
<td>0.022</td>
<td></td>
</tr>
<tr>
<td><em>Diplococcus pneumoniae</em></td>
<td>3,5-diCH$_3$O-TQ</td>
<td>0.016</td>
<td>Glock <em>et al.</em> (1945)</td>
</tr>
<tr>
<td><em>Lactobacillus casei</em></td>
<td>2,3-diCl-1,4-NQ</td>
<td>0.088</td>
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<tr>
<td></td>
<td>MD</td>
<td>0.116</td>
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<tr>
<td><em>Mycobacterium phlei</em></td>
<td>2-OH-3,6-diBr-1,4-NQ</td>
<td>0.12</td>
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</tr>
<tr>
<td></td>
<td>2-NH$_2$-1,4-NQ</td>
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<td>4-NH$_2$-1,2-NQ</td>
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<td>2,8-diNH$_2$-1,4-NQ</td>
<td>0.21</td>
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<td>2-NH$_2$-3-Cl-1,4-NQ</td>
<td>0.24</td>
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</tr>
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<td>2-OH-6-Cl-1,4-NQ</td>
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</tr>
<tr>
<td></td>
<td>MD</td>
<td>0.38</td>
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<td>Reference</td>
</tr>
<tr>
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<td>----------------------------</td>
<td>-----------------------------------</td>
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<tr>
<td></td>
<td>2-OH-3-Cl-1,4-NQ</td>
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</tr>
<tr>
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<td>2,5-diOH-3-Cl-1,4-NQ</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lawsone</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-OH-3-Br-1,4-NQ</td>
<td>0.78</td>
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<td>2-OH-6-Br-1,4-NQ</td>
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<td>Phthiocol</td>
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<td>2-OH-5-NH₂1,4-NQ</td>
<td>1.3</td>
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<tr>
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<td></td>
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<td>Mycobacterium tuberculosis</td>
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<td>Lloyd and Middlebrook (1944)</td>
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<tr>
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<td>Lawsone</td>
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</tr>
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<td>2-OH-3-Cl-1,4-NQ</td>
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<td></td>
<td>MD</td>
<td>0.28</td>
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<td>4-NH₄-1,2-NQ</td>
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<td>2,5-diOH-3-Cl-1,4-NQ</td>
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<td>Lawsone</td>
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<tr>
<td>Staphylococcus aureus</td>
<td>2,6-diCH₂O-p-Q</td>
<td>0.009</td>
<td>Oxford (1942)</td>
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<td>6-CH₃O-TQ</td>
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<td>CH₃O-p-Q</td>
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<td>9,10-PAQ</td>
<td>0.0096</td>
<td>Page and Robinson</td>
</tr>
<tr>
<td></td>
<td>MD</td>
<td>0.0174</td>
<td>(1943)</td>
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Table 5-9 (continued)

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<th>Organism</th>
<th>Inhibitor</th>
<th>Bacteriostatic concentration (mM)</th>
<th>Reference</th>
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<td>3-CH₃-MD</td>
<td></td>
<td>0.054</td>
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<tr>
<td>2,5-diCH₃O-p-Q</td>
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<td>0.06</td>
<td></td>
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<td>Fumigatin</td>
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</tr>
<tr>
<td>DQ</td>
<td></td>
<td>0.61</td>
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</tr>
<tr>
<td>1,4-NQ</td>
<td></td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>2,5-diOH-p-Q</td>
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<td>&gt;0.72</td>
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</tr>
<tr>
<td>TQ</td>
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</tr>
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</tr>
<tr>
<td>p-Q</td>
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<td>&gt;1.84</td>
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<td>0.044</td>
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</tr>
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<td>Vinet (1945)</td>
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<td>3,5-diCH₃O-TQ</td>
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<td>0.081</td>
<td>Glock et al. (1945)</td>
</tr>
<tr>
<td>3,5-diCH₃O-TQH₂</td>
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<td>0.081</td>
<td></td>
</tr>
<tr>
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<td>0.021</td>
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</tr>
<tr>
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<tr>
<td>2-Br-1,4-NQ</td>
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<tr>
<td>Thymoquinone</td>
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</tr>
<tr>
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**Gram-negative bacteria**

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5. QUINONES

Table 5-9 (continued)

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</tr>
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<td>Pseudomonas aeruginosa</td>
<td>TQ</td>
<td>1.0</td>
<td>Kavanagh (1947)</td>
</tr>
<tr>
<td></td>
<td>MD</td>
<td>&gt;2.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3,5-diCH$_2$O-TQ</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>Salmonella typhosa</td>
<td>MD-SO$_3^-$</td>
<td>0.015</td>
<td>Pitzurra and Rossini (1954)</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>2-OH-3,6-diBr-1,4-NQ</td>
<td>0.15</td>
<td>Alcalay (1947 a)</td>
</tr>
<tr>
<td></td>
<td>2-NH$_2$-3-Cl-1,4-NQ</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-NH$_2$-1,4-NQ</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4-NH$_2$-1,2-NQ</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-OH-3-Cl-1,4-NQ</td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-OH-6-Cl-1,4-NQ</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-N(CH$_3$)$_2$-1,4-NQ</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lawsons</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-OH-6-Br-1,4-NQ</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2,5-diOH-3-Cl-1,4-NQ</td>
<td>1.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-OH-3-Br-1,4-NQ</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-OH-5-NH$_2$-1,4-NQ</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Phthiocol</td>
<td>2.6</td>
<td></td>
</tr>
</tbody>
</table>
reliable for several reasons. First, the inhibitory potency will depend on the experimental conditions, particularly the pH, the temperature, and the medium used. For example, the inhibition of *Hemophilus pertussis* growth by *p*-benzoquinone is much reduced in peptone broth relative to a synthetic medium (Grootten and Bezssonoff, 1935), and the activity of 3,5-diCH$_3$O-TQ against a variety of bacteria is reduced 5-fold in serum broth compared to a glucose broth (Glock *et al*., 1945). Zetterberg (1949) studied the effects of menadione and phthiocol on mycobacteria in five different media and found great variation in the bacteriostatic concentrations. Second, the effectiveness will depend on the density of the bacterial suspension, the lowest concentrations being required with the fewest bacteria (Armstrong *et al*., 1943). Third, there is variation in sensitivity between strains; this is seen with staphylococci and streptococci (Barber, 1944) and particularly well with *Brucella melitensis* (Del Vecchio *et al*., 1948). Fourth, the bacteriostatic concentration will vary with the time chosen for the test, and different times for incubation with the quinones were used by different investigators; an interval of 24 hr was selected in most instances. Finally, the concentrations are often determined by serial dilution, so that certain values may be somewhat too high. For these reasons comparisons should not be made between the values given by different investigators, but the relative activities within a particular study are probably reliable, and hence some information can be obtained on the effects of structural alterations on the antibacterial potency.

**Concentration-Action Relations and Biphasic Effects**

The effects of *p*-benzoquinone on the growth of four species of bacteria are shown in Fig. 5-8, and the effects of a variety of quinones on the growth of *Pseudomonas aeruginosa* are shown in Fig. 5-9. One notes that there is commonly a stimulation of growth at lower concentrations. The degree of stimulation may depend on the medium used, since the growth of *Pseudomonas* is accelerated maximally around 23% by *p*-benzoquinone in meat-peptone medium (Fig. 5-8) and only 7% in synthetic medium (Fig. 5-9). The stimulation may be related to reaction with SH groups, inasmuch as such biphasic effects are often seen with SH reagents, or to participation of the quinone in the bacterial metabolism. There seems to be no obvious correlation between the reactivity with SH groups and the ability to stimulate growth, not is there a clear relation to the redox potentials of the quinones. The marked stimulating action of 2-chloro-3-hydroxy-1,4-naphthoquinone is interesting but impossible to explain. It is also evident that the slope of the concentration-action curve for this compound is greater than for most of the other quinones, so that it completely inhibits growth at a lower concentration than any of the other quinones tested. It would be valuable to have data on the effects of these quinones on bacterial res-
Fig. 5-8. Inhibition of bacterial growth by p-Q in meat-peptone medium. (From Hoffmann-Ostenhof and Fellner-Feldegg, 1949 b.)

Fig. 5-9. Inhibition of the growth of Pseudomonas aeruginosa in synthetic medium by various quinones and naphthoquinones: (1) p-benzoquinone; (2) chloranil; (3) toluquinone; (4) thymoquinone; (5) 1,2-naphthoquinone; (6) 1,4-naphthoquinone; (7) lawsone; (8) phthiocol; (9) 2-Cl-3-OH-1,4-NQ; (10) menadione. (From Hoffmann-Ostenhof and Fellner-Feldegg, 1949 b.)
piration. Quinones often exhibit a diphasic action on respiration (see page 488), but information on bacteria is lacking.

Susceptibilities of Gram-Positive and Gram-Negative Bacteria

Geiger (1946) stated that the quinones are 50–200 times more inhibitory to gram-positive than to gram-negative bacteria, and similar differences have been observed by many workers. It is evident in the curves of Fig. 5-8. Actually the difference between the gram-positives and gram-negatives may not be as great as Geiger estimated, although in his experiments an average activity ratio near 60 was obtained (comparing Staphylococcus aureus and Bacillus subtilis with Escherichia coli and Aerobacter aerogenes), since an average activity ratio of only 2.3 can be calculated in the work of Alcalay (1947 a) (comparing S. aureus, Mycobacterium phlei, and Mycobacterium tuberculosis with E. coli and Shigella flexneri). The data of Kavanagh (1947) provide an intermediate activity ratio of near 23 (comparing S. aureus, B. subtilis, Bacillus mycoides, and M. phlei with E. coli, Klebsiella pneumoniae and Pseudomonas aeruginosa). The variance in the ratios may be due in part to the media used for the growth of the various organisms, and possibly to some extent to the different types of quinone used (e.g., Geiger tested mainly benzoquinones and Alcalay mainly naphthoquinones). It is not immediately clear why Alcalay found so low a sensitivity for S. aureus. For example, six other workers have reported a mean value of 0.029 mM (0.01–0.058 mM) for menadione, whereas Alcalay reports 0.71 mM. Yet his values for E. coli are fairly comparable to the results of others. In any event, gram-positives are generally more sensitive than gram-negatives and it is interesting to speculate whether this implies that the quinones interfere with amino acid accumulation or utilization. One also wonders if this indicates an action by a mechanism similar to that for penicillin; it would be worthwhile to determine if resistance to the quinones could be induced, and if such resistant organisms would also be resistant to penicillin.

Relation of Growth Inhibition to Structure

There is usually no marked difference in the potencies of the quinone and hydroquinone forms (see accompanying tabulation), and this is probably due to the long periods of incubation allowing interconversion; indeed, it is rather surprising that differences are observed at all under aerobic conditions. It is difficult to formulate consistent rules correlating potency with addition of groups to the basic quinone nuclei, since not only do different bacteria behave differently but there is often quite marked variability between the results of different workers. This may be illustrated in the com-
5. QUINONES

<table>
<thead>
<tr>
<th>Organism</th>
<th>Quinone</th>
<th>Potency ratio (Q/QH₂)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. aerogenes</td>
<td>p-Q</td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td>TQ</td>
<td>1.11</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>p-Q</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>TQ</td>
<td>0.48</td>
</tr>
<tr>
<td>E. coli</td>
<td>p-Q</td>
<td>31.9</td>
</tr>
<tr>
<td></td>
<td>TQ</td>
<td>1.95</td>
</tr>
<tr>
<td></td>
<td>3,5-diCH₃O-TQ</td>
<td>&gt;1</td>
</tr>
<tr>
<td>S. aureus</td>
<td>p-Q</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>TQ</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>3,5-diCH₃O-TQ</td>
<td>1.00</td>
</tr>
<tr>
<td>Staphylococcus sp.</td>
<td>p-Q</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>TQ</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>MD</td>
<td>1.00</td>
</tr>
<tr>
<td>S. pyogenes</td>
<td>2,5-diCH₃O-p-Q</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>2,6-diCH₃O-p-Q</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Comparison of menadione with 1,4-naphthoquinone (see accompanying tabulation). Menadione is more active in some organisms and less active in others, and for S. aureus both situations have been reported. Such results,

<table>
<thead>
<tr>
<th>Organism</th>
<th>Potency ratio (MD/1,4-NQ)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. aerogenes</td>
<td>0.43</td>
<td>Geiger (1946)</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>8.7</td>
<td>Geiger (1946)</td>
</tr>
<tr>
<td>E. coli</td>
<td>0.43</td>
<td>Geiger (1946)</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>Marrian et al. (1953)</td>
</tr>
<tr>
<td>M. tuberculosis</td>
<td>0.39</td>
<td>Lloyd and Middlebrook (1944)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>0.55</td>
<td>Del Vecchio et al. (1946)</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>Marrian et al. (1953)</td>
</tr>
<tr>
<td></td>
<td>2.8</td>
<td>Geiger (1946)</td>
</tr>
<tr>
<td></td>
<td>3.6</td>
<td>Page and Robinson (1943)</td>
</tr>
<tr>
<td>Staphylococcus sp.</td>
<td>2.5</td>
<td>Armstrong et al. (1943)</td>
</tr>
</tbody>
</table>

taken in conjunction with the several possible mechanisms by which these quinones can inhibit growth, are not susceptible to accurate analysis. A very similar picture is presented in comparing tolquinone with p-benzoquinone, potency ratios (TQ/p-Q) from 0.5 to 3.2 being observed; furthermore, for E. coli a ratio of 0.57 was reported by Cooper and Mason
(1927) and a ratio of 2.4 by Geiger (1946). Thus the effect of adding a methyl group to either \( p \)-benzoquinone or 1,4-naphthoquinone cannot be characterized in terms of a consistent change in the potency.

The following structure-activity generalizations may be made but should be considered as provisional only.

(A) The progressive substitution of \( p \)-benzoquinone with methyl groups leads at first to an increased potency and then a decreased potency. Geiger (1946) finds the relative potencies for \( p \)-Q:CH\(_3\)-p-Q:diCH\(_3\)-p-Q:triCH\(_3\)-p-Q to be 1:2.3:3.0:0.86 for four bacteria, but additions of a fourth methyl group to form duroquinone brings about a marked divergence, in that gram-positive organisms are quite susceptible (bacteriostatic concentration 0.03 mM) while gram-negative organisms are resistant (bacteriostatic concentration > 6.1 mM). This might be interpreted to indicate that the inhibition of gram-negative bacteria involves reaction with \( \text{SH} \) groups, whereas this is not so in the case of gram-positive bacteria; on the other hand, it might also indicate the importance of the semiquinone form for the inhibition of gram-positive bacteria.

(B) The progressive substitution of \( p \)-benzoquinone with chlorine atoms also seems to augment the activity at first but later the potency falls. The relative activities of the series \( p \)-Q:diCl-p-Q:triCl-p-Q:tetraCl-p-Q are 1:1.71:0.74:< 0.37 from the results of Geiger (1946). Chloranil is rather inactive on both gram-positives and gram-negatives.

(C) Methoxy groups apparently increase the activity in the \( p \)-benzoquinone series — e.g., diCH\(_3\)O-p-Q's are invariably more potent than \( p \)-Q by factors of 1.6 to 30 (mean 7.3) — but decrease the activity when introduced into toluquinone — e.g., the potency ratio for 3,5-diCH\(_3\)O-TQ:TQ averages 0.30 for six bacteria, and only in \( S. \) aureus is the methoxylated derivative more active.

(D) Substitution in the 2-position of 1,4-naphthoquinone usually reduces the activity if the groups are hydroxyl, chlorine, or bromine. We have seen that a methyl group, forming menadione, can alter the potency in either direction. Inasmuch as 2-NH\(_2\)-1,4-NQ is 4.8 times as potent as menadione on four bacteria (Alcalay, 1947 a), it is likely that the amino group increases the activity.

(E) The effects of substitutions in the 2- and 3-positions of 1,4-naphthoquinone are important as evidence for the role of \( \text{SH} \) reaction in the inhibitory mechanism. When menadione is substituted at the 3-position with a hydroxy group (to form phthioocol), methyl group, or methoxy group, the activity is diminished. A number of 2,3-disubstituted 1,4-naphthoquinones are fairly active, but in all cases there is a halogen atom in the 3-position, and we have seen that this does not interfere with \( \text{SH} \) reaction, so that the data from this group are not pertinent.
(F) The introduction of anionic groups, such as sulfonate, phosphate, or carboxylate, lowers the activity, possibly through impeding penetration into the bacteria, although these groups could also, if they remain on the rings, electrostatically interfere with SH reactions or inductively modify the quinone reactivity.

**Mechanisms of Antibacterial Action**

The bacteriostatic action of the quinones was believed by Cooper (1913) to be associated with the reaction of the quinones with amino or imino groups on the bacterial proteins, since she had demonstrated that the active form is the quinone and that the action is unlike that of the phenols, which are much less potent. Later, Cooper and Haines (1928) altered the original concept because they found \( p \)-benzoquinone to be more inhibitory than toluquinone (see accompanying tabulation), and yet the two act

<table>
<thead>
<tr>
<th>Organism</th>
<th>Potency ratio ((p-Q/TQ))</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella typhosa</em></td>
<td>13</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>8</td>
</tr>
<tr>
<td><em>Proteus vulgaris</em></td>
<td>6</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>5</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>4</td>
</tr>
</tbody>
</table>

similarly on proteins \((p-Q/TQ\) potency ratio is 1.5). On the other hand, the potency or reactivity ratios for the amino acids often fall in the range 3-6 (see page 441). It was thus felt that reaction of the quinones with intracellular amino acids might be responsible for the growth inhibition. More recent results comparing \( p \)-benzoquinone and toluquinone have not provided so marked a difference in potency, and in general the latter seems to be the more potent (even in *E. coli*, \( p-Q/TQ\) ratios of 0.42 and 1.74 have been obtained) (Table 5-9). Although intracellular amino acids are undoubtedly important in bacterial growth, it is doubtful that most of the quinones in bacteriostatic concentrations react appreciably with them. Hilpert (1925) also proposed a mechanism involving the oxidation of protein groups, and Marini-Bettolo and Del Pianto (1947) claimed a parallel between the ability of various quinones to dehydrogenate amino acids and the bacteriostatic activity.

Several workers have suggested that the inhibition of bacterial growth by many substances is related to the redox potential. It is usually assumed that a cell can operate effectively only within a certain redox range and that certain introduced substances can shift the cell out of this range.
Page and Robinson (1943) studied 14 quinones against *S. aureus* and *E. coli* and found no significant correlation with redox potential. They pointed out that all highly active quinones have \( E_0' \) values in the range from \(-0.10\) to \(+0.15\) v but that not all the quinones in this range are potently bacteriostatic. It was felt that the redox potential may be of some importance but is certainly not the dominant factor. One might add that it is not necessarily justifiable to compare all types of quinone in this way since they probably do not all act by the same mechanism; thus comparison of benzo- and naphthoquinones may not be valid. If one considers only the benzoquinones in their work, an increase in activity occurs as the potential drops from \(+0.29\) to \(+0.13\) v, and then decreases as the potential drops lower. However, examining other data (Geiger, 1946; Hoffmann-Ostenhof and Fellner-Feldegg, 1949 b, and others), no correlation between activity and redox potential is evident. The suggestion of Geiger (1946) that the semiquinones are the active forms seems to have little support and there is no obvious correlation between activity and semiquinone stability; indeed, duroquinone, which is particularly stable in the semiquinone form, is often quite a weak inhibitor.

Bacterial oxidative metabolism involves natural quinones and it is conceivable that inhibitory quinones displace or compete with them. Phthiocol was originally isolated from mycobacteria in 1933, and Kimler (1950) examined menadione as an analog of phthiocol to determine if it would inhibit mycobacterial growth. However, he did not determine if the inhibition can be overcome by phthiocol or any vitamin K. Odier (1949) could detect no reduction of the inhibitory action of 2-hydroxy-3-chloro-1,4-naphthoquinone on *M. phlei* by phthiocol. It is true that the mycobacteria are rather resistant to phthiocol, and that growth is often stimulated by this substance, but there is no good evidence that it participates in the metabolism of this organism. In view of the recent discoveries in this field, it appears that the time is ripe for a more detailed examination of this question.

Reaction of the quinones with SH groups has been postulated as a mechanism for the bacteriostasis. Colwell and McCall (1945) found that the inhibition of the growth of *E. coli* by menadione, 3-methylmenadione, and 6-methylmenadione can be reduced by thioglycolate, mercaptoethane, and cysteine. The inhibition by 3-methoxymenadione, however, was not affected. It should be clear by now that antagonism by thiols shows only that the quinones can react with SH groups but does not prove that the reaction occurs or is important in the bacteria. Geiger (1946) claimed that thiols antagonize the actions of quinones on gram-negative bacteria but not on gram-positive bacteria, but this was not confirmed by Hoffmann-Ostenhof and Fellner-Feldegg (1949 b), who found thiols to reduce the inhibition on all bacteria. We have noted that occasional results favor the
necessity for a reactive double bond, but in Table 5-9 there are numerous instances which do not conform to a theory based entirely on SH group reaction. For example, 3-methylmenadione is about 12 times more potent than 1,4-naphthoquinone in inhibiting the growth of S. aureus (Page and Robinson, 1943), and the inhibitions exerted by trimethyl-p-benzoquinone, duroquinone, chloranil, and others cannot be attributed to reaction with SH groups. It is quite possible that inactivation of intracellular thiols or reaction with membrane, mitotic, or enzyme SH groups is involved in certain instances, but other factors are obviously important. Accurate correlations here are impeded by the lack of information on the relative rates at which the various bacteriostatic quinones react with thiols or protein SH groups. Another unknown factor is the possible metabolism of certain quinones by bacteria, some perhaps being inactivated rather rapidly so that their true potency is obscured.

It is interesting that menadione is able to induce the appearance of variants in bacterial cultures. Colwell (1946) noted that stable small colony variants of E. coli are produced regularly by exposure to 0.018–0.145 mM menadione, this being well below the bacteriostatic concentration. These cells ferment sugars slowly, have a depressed acid production, and grow slowly. She felt that this might result from selection of these forms from the original culture, but there was no evidence for this. Clark et al. (1950) reported that menadione can induce penicillin- and streptomycin-resistant strains in S. aureus (see accompanying tabulation). Selection was ruled out here by the fact that menadione also produces a number of other mutations (e.g., small colony variants and some unable to ferment mannitol). The mechanism for this action is not understood but it might imply some action of menadione on chromosomal or enzyme-forming systems, and this could have bearing on growth inhibition.

There has been very little work on the metabolic effects of the quinones on bacteria and it is thus impossible to determine whether the growth inhibition has a metabolic basis. Inspection of Table 5-2 shows that only

<table>
<thead>
<tr>
<th>Menadione (mM)</th>
<th>Number of mutants resistant to:</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Penicillin</td>
<td>Streptomycin</td>
</tr>
<tr>
<td>0</td>
<td>1.4</td>
<td>1.3</td>
</tr>
<tr>
<td>0.0028</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>0.0057</td>
<td>3.0</td>
<td>2.1</td>
</tr>
<tr>
<td>0.171</td>
<td>8.2</td>
<td>3.2</td>
</tr>
<tr>
<td>0.342</td>
<td>21.0</td>
<td>4.6</td>
</tr>
<tr>
<td>0.684</td>
<td>No growth</td>
<td>No growth</td>
</tr>
</tbody>
</table>
two bacterial enzymes have been shown to be potently inhibited — there is almost complete inhibition of the NAD(P)H:nitrate oxidoreductase from Achromobacter fischeri by 0.03 mM menadione and of the pyruvate oxidase from Neisseria gonococcus by 0.03 mM p-benzoquinone — but actually very few enzymes have been examined. Hoffmann-Ostenhof and collaborators have found little or no correlation between growth inhibition and the effects on various enzymes, but unfortunately most of these enzymes are unrelated to metabolic systems of importance to bacteria. Menadione is able to uncouple oxidative phosphorylation in particulate fractions from bacteria (see page 479) and protein synthesis is depressed, but it is not known if these actions are related in any way to bacteriostasis. In no case has a series of quinones been tested on both growth and some enzyme or metabolic activity in order to establish a possible correlation. Growth appears to be suppressed more readily than is respiration, from the meager results available. Thus Vogler et al. (1942) found that p-benzo-hydroquinone prevents growth of Thiobacillus thiooxidans at 0.01–0.1 mM, these concentrations presumably not altering respiration. Finally, there is no evidence to suggest a common mechanism of growth inhibition, and it is perhaps preferable at the present time to assume that various types of quinone act primarily in different ways.

Effects on Bacterial Infections

Quinones were tested for their germicidal and antiseptic activity many years ago but they have not proved to be clinically useful, due mainly to the fact that bactericidal concentrations are much higher than bacteriostatic concentrations. For example, p-benzoquinine inhibits the growth of S. aureus at 0.032 mM but 2 mM is required for a cidal action (Yamada and Yanagita, 1957). Although p-benzoquinone was the most effective bactericidal substance examined by Thalhimer and Palmer (1911), having a phenol coefficient of 160 against Salmonella typhosa, it still required 0.58 mM to be disinfectant after 15-min exposure. The results of the use of the quinones in the treatment of systemic infections have been generally negative. Thus 3,5-dimethoxytoluquinone, despite its effectiveness against certain bacteria in vitro, does not protect mice inoculated with streptococci (Barber, 1944) or staphylococci (Glock et al., 1945). Even the antibiotic quinones, such as spinulosin or fumigatin, have not been found able to protect mice against pneumococci (Huisman, 1950) and have not proved clinically useful. Two positive results have been reported but have not been adequately followed up. Alcalay (1947 b) administered 2-hydroxy-3-chloro-1,4-naphthoquinone orally to guinea pigs with tubercular infections, and found a reduction of the weight loss and a prolonged survival of the animals. Del Vecchio (1948 a) tested many quinones in vitro against Brucella and found 6-hydroxymenadione to be particularly...
active, the bacteriostatic concentration being 0.0047 mM. As the sodium sulfonate this compound was designated "antibrucellin" and found to be well tolerated by various routes. Successful treatment of 25 of 29 cases of human brucellosis was reported (Del Vecchio, 1948 b).

**EFFECTS ON FUNGI AND YEASTS**

Certain quinones have been widely used as agricultural fungicides for the past 30 years. Halogenation markedly increases the antifungal potency of quinones and the two most commonly used compounds commercially are chloranil (Spergon) and 2,3-dichloro-1,4-naphthoquinone (Dichlone, Phygon). They are applied to seeds, seedlings, and adult plants to control various rusts, rots, mildews, wilts, and blights due to fungi in legumes, fruit trees, ornamental plants, and various vegetables. At the proper concentrations they will usually suppress the fungi without damaging the plants, but occasionally they may discolor fruits or leaves. The quinones have not been found to be useful in clinical mycoses, mainly because they are irritant to human tissues. Some results on the inhibition of fungal growth are summarized in Table 5-10. Two facts are immediately evident: (1) fungi and yeasts are very susceptible to certain quinones, particularly the chlorinated derivatives, and are often inhibited at concentrations of 0.0001–0.001 mM; and (2) the relative potencies against fungi and yeasts are quite different from those observed in the inhibition of bacterial growth. As is frequently the case with bacteria, growth stimulation is usually observed at low concentrations of certain quinones. It has been shown that p-benzohydroquinone can serve as the carbon source for the growth of *Aspergillus oryzae* (Tamiya, 1932), and that *Aspergillus niger* metabolizes the hydroquinone to keto acids (since β-ketoadipate accumulates in the presence of arsenite), which are decarboxylated and oxidized (Friedrich, 1956), but whether such utilization is related to the growth stimulation is not known. The fungi are similar to bacteria in that they are killed only by much higher concentrations than are required to inhibit the growth; differences around 100-fold in the cidal and static concentrations being common. It must also be remembered that fungi often possess active enzyme systems for the degradation of substances not attacked by bacteria, and thus destruction of the inhibitors may in some instances have reduced the potency, the figures in the table thus being occasionally too high. Rich and Horsfall (1954) suggested that one factor determining the susceptibilities of fungi to both polyphenols and quinones might be the content of polyphenol oxidase, and presented some evidence based on a study of 42 compounds that such a correlation may exist. Two other factors controlling the susceptibility of fungi to quinones are the pH (F. G. Smith *et al.*, 1946) and temperature (Molho and Lacroix, 1949). The inhibition of the growth of *Glaucoma piri-*
*formis* is so sensitive to temperature that 0.00057 mM menadione stimulates at 60°-100° and inhibits at 110°-120°, other naphthoquinones exhibiting similar effects. Too little attention has been paid to these factors in determining antifungal potencies.

**Relation of Growth Inhibition to Structure**

There is a wide range of susceptibility between the various fungi to a particular quinone; e.g., some fungi are very sensitive to menadione or 2,3-dichloro-1,4-naphthoquinone, and others are quite resistant, a range of over 1000-fold sometimes being observed. This is in part due to the different experimental conditions,* but one would also expect fungi to exhibit a wide response spectrum since they do not constitute a homogeneous group of organisms. It is evident that some particular activity or mechanism must be associated with certain quinones, inasmuch as they are so much more potent than others for a single type of fungus; i.e., although the quinone structure may be important, other factors markedly modify this activity. It is difficult to make generalizations concerning the relation of potency to structure, and there will always be exceptions to any rule, but a few possible correlations will be mentioned.

(A) In 9 of 10 fungi the quinone is more potent than the corresponding hydroquinone, the relative potency ratio averaging 5. The difference here appears to be greater than for the bacteria.

(B) Addition of chlorine atoms strongly increases the potency. In the benzoquinone series, the relative potency ratios for \( p-Q:diCl-p-Q:tetraCl-p-Q \) are 1:5:13, and in the naphthoquinone series the ratios for \( 1,4-NQ:2-Cl-1,4-NQ:2,3-diCl-1,4-NQ \) are 1:8:80, these figures being very rough approximations but indicative of the magnitude of the effects. Furthermore, 2,3-dichloro-1,4-naphthoquinone is around 25 times more potent than chloranil.

(C) The effects of methylation are not consistent. Toluquinone seems to be generally less potent than \( p \)-benzoquinone, but xyloquinone is more potent, a mean potency sequence of 1:0.64:14 being obtained from limited data. There is so much variation between responses to 1,4-naphthoquinone and menadione that the effect of the 2-methyl group cannot be definitely stated. The potency ratio MD:1,4-NQ runs between 0.077 and 16.8. If anything, menadione is usually somewhat less active. It may be noted that 2-chloro-1,4-naphthoquinone is around 16 times more potent than menadione, although again there is considerable variation.

* One notes that in the studies in which two or more fungi were tested, the sensitivities do not vary nearly as much as between the values given by different investigators.
### Table 5-10

**Inhibition of Fungal and Yeast Growth by Quinones**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Inhibitor</th>
<th>% Growth inhibition</th>
<th>Concentration (mM)</th>
<th>Reference</th>
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<td>Conc.</td>
<td>IC₅₀</td>
<td>Ref.</td>
</tr>
<tr>
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<td>$p$-QH₂</td>
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<td>2,3-diCl-5-OH-1,4-NQ</td>
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<td>2,3-diCl-1,4-NQ</td>
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<td>9,10-PAQ</td>
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<td>Chloranil</td>
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<tr>
<td></td>
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<td>0.092</td>
<td>Woolley (1945 a)</td>
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</tr>
<tr>
<td>Organism</td>
<td>Inhibitor</td>
<td>% Growth inhibition</td>
<td>Concentration (mM)</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------</td>
<td>--------------------</td>
<td>---------------------</td>
<td>--------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>Naphthazarin</td>
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<tr>
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<tr>
<td>p-Q</td>
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<tr>
<td>1,2-NQ</td>
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<td>0.047</td>
<td></td>
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</tr>
<tr>
<td>TQ</td>
<td>50</td>
<td>0.10</td>
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</tr>
<tr>
<td>4-CH₃O-TQ</td>
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<td>2,6-diCl-p-Q</td>
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<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2,6-diCH₃O-p-Q</td>
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<td>0.22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MD</td>
<td>11</td>
<td>0.051</td>
<td></td>
<td>Kiesow (1960 b)</td>
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<tr>
<td>5-CH₃O-TQ</td>
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<td>0.067</td>
<td></td>
<td>Huisman (1950)</td>
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<tr>
<td>Compound</td>
<td>Dose (µg/mL)</td>
<td>Concentration (%)</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------</td>
<td>------------------</td>
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<td></td>
</tr>
<tr>
<td>2,6-diCH₃O-ortho-Q</td>
<td>50</td>
<td>0.41</td>
<td>Kligman and Schnee (1960)</td>
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<tr>
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<td>50</td>
<td>0.60</td>
<td>Kligman and Schnee (1960)</td>
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<td>2,5-diCH₃O-para-Q</td>
<td>50</td>
<td>0.43</td>
<td>Kligman and Schnee (1960)</td>
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</table>

*Inhibition of germination.*
(D) Methoxy groups generally lower the activity in the naphthoquinone series and, although data are not available, the methoxylated benzoquinones are never very active.

(E) Hydroxylation of 1,4-napthoquinone in either the 2- or 5-position leads to loss of activity, these compounds having only one tenth–one fifth the activity of menadione, which is roughly equivalent to 1,4-napthoquinone.

There seems to be no question but that the quinone structure is of importance in the antifungal action, since the corresponding nonquinonoid compounds are usually not as inhibitory. González (1945) showed that β-methylnaphthalene is a much weaker inhibitor of Penicillium notatum than is menadione. However, this does not imply that the quinone groups are directly involved in the reaction with the target components of the fungal cells.

Inhibition of Yeast Growth

With respect to sensitivity to the quinones in general, yeasts seem to lie somewhere between the gram-positive and gram-negative bacteria but, according to Woolley (1945 a), are much more inhibited than any bacteria or fungus by 2,3-dichloro-1,4-napthoquinone. Such high sensitivity was not noted by Hoffmann-Ostenhof and Fellner-Feldegg (1949 a), but these discrepancies are undoubtedly due to the different conditions under which the yeast was grown. The most potent inhibitors of yeast in the survey of the latter workers are naphthazarin and phthiocol, which are only weakly inhibitory to most bacteria, whereas the methoxy derivatives appear to be more effective against bacteria than yeast. The inhibitions of yeast α-glycerophosphatase (Hoffmann-Ostenhof and Putz, 1948) and yeast proteinase (Hoffmann-Ostenhof and Moser, 1948) were claimed to exhibit some correlation with growth inhibition, but this is very doubtful (see Table 5-2). The quinones interfere with phosphate uptake by yeast only at high concentrations, 0.1 mM somewhat stimulating uptake and 1 mM inhibiting after 3 hr (Hoffmann-Ostenhof and Kriz, 1950), and have little effect or slightly stimulate lipid synthesis (Hoffmann-Ostenhof and Kriz, 1949 a). Yeast fermentation is generally accelerated by various quinones, except at high concentrations, but there is some depression of respiration at concentrations of 0.01–0.1 mM (Hoffmann-Ostenhof and Kriz, 1949 b). However, there is no correlation between respiratory and growth inhibitions; e.g., 2,3-dichloro-1,4-napthoquinone inhibits less than 2-chloro-1,4-napthoquinone and 1,2-napthoquinone, and to about the same extent as p-benzoquinone, tolquinone, and menadione. Lejhanec et al. (1931) found that p-benzoquinone at 0.0184 mM increases respiration and simultaneously depresses growth. No correlations between the effects on growth and metabolism have been established. Menadione and ferricyanide
together, at concentrations which inhibit only slightly when each is present alone, block growth completely; this was interpreted by Kiesow (1960 b) to mean that the quinone form of menadione is active, since ferricyanide is able to reoxidize menadiol as it is formed, but there are other possible explanations, and even if it is true it does not indicate the mechanism by which menadione inhibits.

Mechanisms of Antifungal Action

The growth of *Phycomyces blakesleeanus* in a synthetic medium is inhibited by menadione, and this inhibition is reversed by the addition of nicotinate, nicotinamide, or various possible precursors of these (e. g., anthranilate, 3-hydroxyanthranilate, indole, kynurenin, and tryptophan) (Schopfer and Boss (1948). When this organism is grown with menadione there occur some interesting changes in the levels of certain cofactors, as determined by analysis of the thalli and medium (Schopfer and Boss, 1949). Some cofactors rise and some fall, and the relationship to menadione concentration is very puzzling (see accompanying tabulation). The interference

<table>
<thead>
<tr>
<th>Substance</th>
<th>% of control level</th>
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</thead>
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<tr>
<td></td>
<td>MD</td>
</tr>
<tr>
<td></td>
<td>0.012 mM</td>
</tr>
<tr>
<td>Nicotinate + nicotinamide</td>
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</tr>
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<td>Lactoflavin</td>
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<tr>
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<tr>
<td>Folate</td>
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</tr>
<tr>
<td>p-Aminobenzoate</td>
<td>Trace</td>
</tr>
<tr>
<td>Biotin</td>
<td>73.1</td>
</tr>
<tr>
<td>Choline</td>
<td>60.5</td>
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</table>

with the synthesis of pyridine nucleotides may be one major mechanism of the growth inhibition, since the most potent reversers are nicotinate, nicotinamide, and NAD, but disturbances in the other cofactors must also play a role. Thiamine was added to the medium and menadione increased its destruction; 49% was lost in the control, 67% in 0.012 mM menadione, and 73% in 0.048 mM menadione. Bisceglie (1951) also reported that 2,3-dichloro-1,4-naphthoquinone at 0.00044 mM or lower markedly inhibits the formation of nicotinate in *Deuterophoma tracheiphila*, a phytopathogenic fungus, and moderately depresses the synthesis of riboflavin and folate. Furthermore, certain amino acids, e. g., arginine, tryptophan, proline, and
asparagine, are able to antagonize the action of the quinone, and this may be related to the synthesis of cofactor. It is unfortunate that this work was not carried further and that this type of investigation is rare in these fields, since it is only by quantitative analysis of the compositional changes occurring during the actions of growth inhibitors that one can approach the site or sites of inhibition.

Woolley (1945 a) studied the actions of 2,3-dichloro-1,4-naphthoquinone on various microorganisms in order to determine if it is an antagonist of vitamin K. Menadione was examined for its ability to reverse the inhibition by 2,3-dichloro-1,4-naphthoquinone. A difficulty lies in the fact that menadione is quite a potent inhibitor itself, depressing growth 50% at 0.0013–0.0016 mM in the case of yeast and *Endomyces vernalis*. However, menadione at concentrations around one tenth of this reduced the inhibition by 2,3-dichloro-1,4-naphthoquinone and this was claimed to be competitive over a limited concentration range. No such inhibition reversals with menadione were seen in the four bacterial species studied. Hoffmann-Ostenhof *et al.* (1947 b) could not confirm this antagonism in yeast, but later some antagonism was observed (Hoffmann-Ostenhof and Fellner-Feldegg, 1949 a). Guerillo-Vinet and Guerillo-Vinet (1948) observed an antagonism by menadione of the growth inhibition of *Fusarium solani* by 2-chloro- and 2-methoxy-1,4-naphthoquinones, and Molho and Lacroix (1950) found the same for *Aspergillus niger*. In the latter case, the inhibition over 10 days by menadione at 0.071 mM was 68%, by 2-chloro-1,4-naphthoquinone at 0.065 mM 95%, and by both together at the same concentrations only 27%. These results are not as easy to interpret as some other antagonisms. For example, if menadione is normally functional in the metabolism of these fungi, what is the mechanism for its inhibition of growth? And why is its inhibition reduced by the chlorinated naphthoquinone? The answer to these and other questions will come only when we have information on the role of naphthoquinones in fungal metabolism, and data on the effects of inhibitory quinones on isolated enzyme systems.

The inhibitions of spore germination and the pyruvate decarboxylase of *Monilinia fructicola* by various naphthoquinones are of the same order of magnitude (see accompanying tabulation), and this suggested to Foote *et al.* (1949) that this enzyme might be a major site of action. The relatively low potency of 1,4-naphthoquinone in inhibiting germination was attributed to its high reactivity, much of it being bound. Despite the discrepancies, this is certainly one of the few convincing correlations that have been made in this field, and furthermore implicates an enzyme of undoubted importance in metabolism. In support of this site of action, one recalls the accumulation of pyruvate in *Fusarium lini* induced by low concentrations of 1,4-naphthoquinone (Maselli and Nord, 1952) (Fig. 5-5). It would be interesting to know more about the reactions of 2,3-dichloro-1,4-naphtho-
quinone with SH groups and the mechanism by which it inhibits the pyruvate decarboxylase. Other attempted correlations with enzyme inhibition, as with amylase, catalase, or polyphenol oxidase (Owens, 1953 a, b) are invalidated by many exceptions; e.g., menadione inhibits fungal growth rather potently but has no effect on amylase, and toluquinone is less than 1/2000 as inhibitory to growth as 2,3-dichloro-1,4-napthoquinone but inhibits amylase more potently. One disturbing note is the fact that 3,4-dichlorotoluene is a moderately potent inhibitor of fungal growth, indicating that more work should be done on chlorinated nonquinonoid compounds.

**EFFECTS ON ALGAE AND PROTOZOA**

Algae are perhaps the most sensitive to the quinones of all organisms, on the basis of the limited data available. 2,3-Dichloro-1,4-naphthoquinone is much more potent than any other substance tested on blue-green algae, being inhibitory to growth at 0.00001 \( \text{m}M \), at which concentration no effect on other aquatic life was noted (Fitzgerald *et al.*, 1952). The growth of *Euglena gracilis* is also potently inhibited by naphthoquinones, but there is often quite marked variation in sensitivity between the normal green form and a white chlorophyll-free form (obtained by treatment with streptomycin) (Table 5-11) (Schopfer and Keller, 1951). The marked differences in the susceptibility ratio for the different quinones points to more than a single mechanism of action. Furthermore, menadione inhibits the white form more potently in the light than in the dark, while the green form is inhibited about equally in light and dark. Nicotinate is a growth inhibitor and adds to the menadione inhibition in the green form, but antagonizes the menadione inhibition in the white form. Of course, the different susceptibilities need not depend on the presence or absence of chlorophyll, but may be related to other simultaneous metabolic changes; nevertheless,
the effects of light on the inhibition may indicate some relationship. The white form may be deficient in nicotinate or the pyridine nucleotides when grown in the light, and thus it may be more susceptible to those quinones interfering with NAD(P) synthesis (if similar relations hold as in some of the fungi) and nicotinate will then be able to reverse the inhibition. Unfortunately the effects of nicotinate on the other inhibitions were not determined.

The screening of compounds to find useful antimalarials led to the discovery of the activity of hydrolapachol but since much more active compounds with longer side chains were soon found (e.g. SN-5949), the actions of these naphthoquinones on *Plasmodium* will be discussed elsewhere (Fieser, 1948). The simpler benzo- and naphthoquinones are relatively inactive (Wiselogle, 1946 b). The motility of *Trypanosoma equiperdum* is first inhibited by 0.18 mM 1,2-naphthoquinone-4-sulfonate, at which concentration glycolysis is depressed 40% and respiration 74% (Meyerhof and Randall, 1948). Lower concentrations can significantly inhibit respiration and glycolysis without affecting the motility. It is able to exert a
delaying action on murine trypanosomiasis. It has recently been found that 4,7-phenanthroline-5,6-quinone is effective in human amebiasis (Car-
ter, 1961). Clearing of the infection for 2 months was seen in 71 of 76 patients given 50 mg 3 times a day for 5 days, and no toxic reactions were noted.

**EFFECTS ON INVERTEBRATES**

Various echinoderms, worms, molluscs, and arthropods when exposed to 3–6 mM p-benzoquinone exhibit an initial period of excitement or augmented activity and excitability,* but this is eventually followed by paralysis (Danilewsky, 1895). Muscle contracture may occur terminally. Coelenterates are apparently more resistant and succumb to concentrations around 10 times higher than for other invertebrates; this may be partly related to the ability of coelenterates to isolate themselves from their environment. Most of the subsequent work has been on worms. Labes (1930) used leech muscle to further his analysis of the site of action of p-benzo-
hydroquinone, and found that oxygen and elevated pH favor activity, so that it was concluded that the quinone is the active form. Concentrations near 0.3 mM under optimal conditions first stimulate the muscle strongly but this soon passes into paralysis. Bergstermann (1944) confirmed these observations and noted that muscle contracture occurs. Both Labes and Bergstermann believed that p-benzoquinone attacks enzymes or coenzymes of the respiratory chain, possibly through their SH groups, but no evidence for this was presented.

Planaria frequently respond to noxious influences by a histolytic fragment-
ation and this occurs upon exposure of the worms to lower concentra-
tions of quinones. Von Bertalanffy et al. (1946) plotted the disintegration times against the concentration of various quinones and found that a break occurs in the curves near 1 mM; below this concentration for p-benzo-
quinone and 1,4-naphthoquinone the action is primarily histolytic and

* Among other things, Danilewsky worked with "abgeschnittene Fühler von Octopus," and one statement not only illustrates the initial effect of p-benzoquinone but well expresses the hazards encountered by invertebrate pharmacologists, "Ein Fühler begann an der Wand des Gefäßes aus dem Wasser herauszukriechen; mit einiger Mühe schob ich denselben zurück ins Wasser, er kroch aber wieder hinauf, und dies wiederholte sich einige Male."
above this it is a tanning or fixative action. Fragmentation is initiated in the posterior region and then spreads over the organism. The quinones fall in the following order of decreasing potency: naphthazarin; 1,4-naphthoquinone; methylnaphthazarin; p-benzoquinone; toluquinone; 1,2-naphthoquinone; 2,6-dimethoxy-p-benzoquinone; xyloquinone; 5-methoxytoluquinone; isonaphthazarin; and lawson. p-Benzoquinone at 0.2 mM kills in around 75 min but disintegration begins in 10 min (Hoffmann-Ostenhof et al., 1948). Schreier (1949) investigated these effects quantitatively and showed them to depend on the quinone concentration and the type of quinone used, so that no general pattern of disintegration can be outlined. However, at the lowest concentrations fragmentation is seen to begin at the head end, while at concentrations of 1–3 mM the primary disintegration is at the tail end, higher concentrations fixing the entire animal in a fragmented condition. p-Benzoquinone at 0.03 mM does not alter motility but slowly induces anterior fragmentation which progresses posteriorly; at 0.09 mM there is some abnormal twisting; as the concentration is raised the movements become more abnormal and exaggerated. Numerous curves for (a) time for beginning of head disintegration, (b) time for beginning of tail disintegration, and (c) time for death were plotted relative to the concentration on log-log graphs, linear relationships generally being observed (Fig. 5-10). The author assumed that the rate of the toxic action is given by an equation of the type

\[ \frac{dL}{dt} = kC^n \]

where \( L \) is the degree of particular toxic effect measured, \( C \) is the quinone concentration, and \( k \) and \( n \) are constants, the latter being determined from the slope of the log-log plots, since

\[ \log t = \log \left( \frac{L}{k} \right) - n \log C \]

Although this type of approach is valuable in quantitatively characterizing potencies and kinetics, the interpretations given for \( n \) in terms of adsorption and other processes cannot be accepted. The values of \( n \) differ for head and tail disintegration, but this can be interpreted in various ways, e. g., different rates of penetration, different degrees of metabolic activity, different mechanisms by which the quinones exert their action in the two regions, and different degrees of operation of the factors determining cohesion.

The effects of quinones on the respiration and glycolysis of Schistosoma mansoni have been discussed (page 487) (Bueding et al., 1947; Bueding and Peters, 1951). Glucose utilization and lactate formation are inhibited more potently than is respiration, and it was felt that glycolytic processes are necessary for the survival of the organism; it was also suggested that certain quinones might be of value therapeutically if they were not too ex-
tensively inactivated in serum. Tests in mice infected with *Schistosoma* showed that menadione, the most effective quinone, has relatively little effect by itself, but can potentiate the action of stibophen (Fuadin) in promoting migration of the organisms from the veins to the liver. Several quinones are cercaricidal at concentrations of 0.2–0.5 mM, but the data are too inaccurate to assign relative potencies (Schreiber and Schubert, 1949). It is likely that the cidal mechanism is entirely different from the suppressive or antiglycolytic actions studied by Bueding and his co-workers. Intestinal *Trichinella spiralis* infections in rats were treated with menadione (most other benzo- and naphthoquinones were shown to be ineffective) by Oliver-González and Bueding (1948), and it was shown that oral administration leads to 81% reduction in the number of adult trichinae in the intestine. There is no correlation between the actions of the quinones on *Schistosoma* and *Trichinella* and the mechanisms appear to be different, according to Bueding.
There is little doubt that quinones can inactivate viruses and inhibit their proliferation; the interesting question is whether they can do this specifically to any degree, that is, without simultaneous severe depression of the host cells. Studies in which virus or phage suspensions are incubated with the quinones and later placed in contact with the host cells indicate the reaction of the quinones with virus or phage protein, and do not provide information on the metabolic reactions associated with the proliferative process. p-Benzoinone at 1 mM, for example, completely inactivates psittacosis virus (Burney and Golub, 1948) and influenza A virus (Wagner, 1951), but this is only an expression of the reaction of the quinone with virus SH groups or the oxidation of such groups; indeed, cysteine not only protects against the quinone but can restore some infectivity in the influenza virus after inactivation. Hall et al. (1951) make a very complete investigation of the effects of eight quinones on ten common phages. Streptococcal phage, enterophage, and coliphage 24B are generally the most susceptible and often are inactivated partially by concentrations as low as 0.003–0.02 mM, although complete inactivation requires concentrations greater than 0.3 mM in all cases. Toluquinone and 5-methoxytoluquinone are the most potent inactivators, while the naphthoquinones are not very effective; menadione, for example, at 2.8 mM has no effect on four phages and only moderately inactivates the others, whereas toluquinone inactivates 50% at a mean concentration of around 0.15 mM. Since the phages were incubated with the quinones for several hours, the inactivation does not imply a rapid reaction with phage protein, and it is only surprising that some of the quinones have so little effect on phages which from other data contain SH groups. Sery and Furgiuele (1961) have more recently conducted similar studies on the herpes simplex virus, except that the incubation interval was only 1 hr (see accompanying tabulation). In contrast to the

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>% of virus inactivated</th>
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<tbody>
<tr>
<td></td>
<td>0.001 mM</td>
</tr>
<tr>
<td>1,4-NQ</td>
<td>78</td>
</tr>
<tr>
<td>1,2-NQ</td>
<td>68</td>
</tr>
<tr>
<td>p-Q</td>
<td>0</td>
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<tr>
<td>5-CH₄-TQ</td>
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<td>9,10-AQ</td>
<td>—</td>
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<tr>
<td>tert-Butyl-p-QH₂</td>
<td>—</td>
</tr>
<tr>
<td>MD</td>
<td>—</td>
</tr>
<tr>
<td>1,2-NQ-4-SO₃</td>
<td>—</td>
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</tbody>
</table>
phages, this virus is fairly susceptible to naphthoquinones (Hall et al. did not test 1,2-NQ or 1,4-NQ), and in fact 1,2-naphthoquinone and 1,4-naphthoquinone are more potent inactivators than Hg++ or other SH reagents, indicating some very reactive group on the virus protein. However, none of the quinones was found to be effective in treating corneal herpes infections in rabbits.

The few reports on virus proliferation, both in vitro and in vivo, all agree that the quinones are quite potently inhibitory and that a surprising selectivity may be observed. R. L. Thompson (1947) demonstrated that p-benzoquinone suppresses the proliferation of vaccinia virus in embryo tissue culture around 35% over a period of 4 days at a concentration of 0.00092 mM, while at 0.092 mM proliferation is essentially stopped. p-Benzoquinone is much less effective, probably because the partial anaerobiosis prevents its full conversion to the quinone. Menadione is inactive at 0.058 mM but strongly inhibitory at 0.58 mM. If embryo tissue cultures are exposed to 1 mM p-benzoquinone for 1 hr and then inoculated with psittacosis virus, the cultures grow well but proliferation of the virus is markedly reduced, indicating that some action is exerted on the host cells which interferes with their ability to support virus growth (Burney and Golub, 1948). A clear demonstration of selectivity is found in the work on T2 coliphage by Czekalowski (1952). Incubation of the phage with 1 mM 2-OH-1,4-naphthoquinone, 5.5 mM toluquinone, or 50 mM p-benzoquinone has no effect on subsequent intrabacterial proliferation, but similar treatment of the bacteria completely prevents phage multiplication. However, when the phage is added with the quinone to the bacterial suspension, it was claimed that these quinones reduce phage development without affecting the reproduction of the host bacteria, although no data are given.* These limited studies indicate interesting possibilities for the selective antiviral activities of the quinones, but the significance of this and the mechanisms involved have not been explored.

A very selective action of several hydroquinones on type 2 poliomyelitis virus proliferating in cultures of monkey testicular tissue was demonstrated by Kramer et al. (1955) in a search for possible chemotherapeutic agents. Of the numerous compounds tested, substances which can be oxidized to

* It is somewhat unexpected that the high concentrations of these quinones would be without effect on E. coli since the results of other investigators summarized in Table 5-9 show the quinones used here to be bacteriostatic at concentrations less than one tenth those claimed to be without effect by Czekalowski. Perhaps the difference is due to the times over which the bacterial proliferation was determined. It is not very clear how Czekalowski evaluated the effects on the bacteria, since the only data given for this type of experiment, illustrating the effect of fluoride, cover a period of only 60 min, during which time it would be difficult to determine if there were an effect on the bacteria.
quinonoid forms are particularly effective (Table 5-12). The \textit{para} are more selective than the \textit{ortho} isomers. Phenol is ineffective and the addition of most groups does not create virustatic agents, but the addition of a hydroxy or amino group in the proper position brings out an interesting and perhaps useful specificity. \textit{p}-Aminophenol and toluhydroquinone act on the virus only outside the cells, while the rest seem to penetrate into

\begin{table}
\centering
\caption{Effects of Hydroquinones and Aminophenols on Poliomyelitis Virus in Tissue Culture}
\begin{tabular}{llll}
\hline
Compound & Highest concentration nontoxic to cells (mM) & Lowest concentration inhibitory to virus (mM) & Inhibitory index \\
\hline
\textit{p}-Benzhydroquinone & 73\textsuperscript{a} & 0.134\textsuperscript{b} & 533 \\
Toluhydroquinone & 16.1 & 0.12\textsuperscript{b} & 133 \\
Phenyl-\textit{p}-benzo-hydroquinone & 5.4 & 0.081\textsuperscript{b} & 66 \\
Dichloro-\textit{p}-benzo-hydroquinone & 11.2 & 0.167 & 66 \\
\textit{p}-Aminophenol & 2.3 & 0.074\textsuperscript{b} & 33 \\
Catechol & 4.5 & 0.546 & 8 \\
\textit{o}-Aminophenol & 18.4 & 18.4\textsuperscript{b} & 1 \\
\hline
\end{tabular}
\end{table}

\textsuperscript{a} Highest concentration tested.
\textsuperscript{b} Lowest concentration tested.

the cells and act there. That all of these compounds may not be acting by the same mechanism, or on the same site, is indicated by the fact that glutathione antagonizes toluhydroquinone, cysteine antagonizes phenyl-\textit{p}-benzhydroquinone, and various amino acids (serine, threonine, and hydroxyproline) antagonize \textit{p}-benzhydroquinone and \textit{p}-aminophenol. It is surprising that a substance such as \textit{p}-benzhydroquinone or toluhydroquinone can be so selective on virus proliferation, and so little damaging to the host cells.

**DISTRIBUTION AND METABOLISM**

As with any aspect of the quinones, it is difficult to present a unified picture inasmuch as there are several basic types of quinone and they are
distributed and metabolized quite differently. These differences, and in most cases even the possibility of metabolic alteration of the quinones, have been mostly ignored. The reports on the metabolism of the quinones may not be uniform but there is much to indicate that most quinones are not as stable as many investigators believe, and that this must be taken into account particularly in experiments of long duration.

Binding to Serum

Since most quinones react readily with proteins, it is not surprising that the presence of protein reduces the inhibitory activity. It has been reported several times that protein-containing media diminish the growth-inhibiting activity. It is obvious from such studies that the effective concentrations reported must be considered with respect to the conditions under which the experiments were done. The marked binding to human serum proteins is a major factor in limiting the clinical effectiveness of many quinones. We have noted that the inhibition of schistosomal glycolysis by menadione is reduced by serum, approximately 5 times the concentration being required in human serum relative to a salt medium (Bueding, 1950), and such binding of menadione not only must play an important role in any in vivo anthelmintic effects, but will in general suppress all the pharmacological actions of menadione. Many of the antimalarial naphthoquinones, which are quite effective in treating animal infections, are valueless clinically because of the tight complexing with the serum proteins in man. This factor also makes it very difficult to compare the effects of quinones on isolated enzymes with the effects observed on living tissues, since even under the simplest conditions of a protein-free medium, there is much so-called non-metabolic protein with which the quinones may react. The thiols in sera and experimental media likewise contribute to the reduction in effective quinone concentration, and limit the distribution into cells and tissues.

Permeability of Cells to Quinones and the Effects of pH

Very little is known about the rates of penetration of the quinones into cells and we have only indirect evidence. For example, succinate oxidase extracted from heart muscle is quite sensitive to various naphthoquinones but within heart slices it is resistant and inhibited only after prolonged exposure (Ball et al., 1947). This was interpreted as indicating a permeability factor, but actually the difference may be attributed to a variety of factors, such as protection of the enzyme intracellularly by substrate or thiols, or different physical states of the enzyme. Some failures to find evidence for penetration may be due to binding or reaction with extracellular substances. The low levels of menadione in the tissues following intramuscular administration to mice (Solvonuk et al., 1952) may be related primarily
to the binding by serum discussed in the previous section. One would certainly expect most quinones to enter cells readily since they are uncharged over the physiological pH range, although some hydroxy naphthoquinones are ionized to varying degrees (see page 428), and they possess lipophilic regions of some extent, and indeed much work has indicated a rapid action on cells. However, evidence from rates of action on cellular functions is not good since in most instances we do not know if the site of action is really intracellular.

The effects of pH on the activities of the polyphenols are related mainly to the rates at which oxidation to quinones occurs, so that, as we have discussed in several places, the hydroquinones usually act more rapidly and to a greater degree as the pH is raised. There is no evidence that rate of penetration or ionization of the phenols is involved. Since most of the quinones do not ionize in the physiological pH range, it is likely that any pH effects observed must be due to factors other than variation in a penetrable form. The effectiveness of most quinones on cellular processes decreases as the pH is raised: menadione at 0.006 mM has no effect on *Achromobacter* luminescence at pH 7.3 but inhibits completely at pH 4.5 (McElroy and Kipnis, 1947), phthiocol is less effective in stimulating algal respiration as the pH increases (Gaffron, 1945), various quinones cease to affect the phosphate uptake of yeast when the pH is raised (Hoffmann-Ostenhof and Kriz, 1948), and naphthoquinones are less potently antibacterial at pH 7.3 than at 6.0 (Marrian et al., 1953). The only exception to this is the moderately greater inhibition of glycolysis in ascites carcinoma cells by *p*-benzoquinone as the pH is raised from 6.0 to 7.3 (Holzer et al., 1956). The respiration of yeast is inhibited by toluquinone to approximately the same degree at all pH’s from 5.2 to 8.0, although there may be a slight maximum around neutrality (Hoffmann-Ostenhof and Kriz, 1949 b). Several factors must be involved in these pH effects, including the alterations in membrane permeability, changes in the rate of reaction with SH of NH₂ groups, the stability of the quinones, and the variation in the redox potentials with pH. In any event, the data from pH studies provide no direct evidence for evaluating rates of penetration.

The general stability of the quinones, the reactions with thiols and amines, and the oxidation-reduction reactions occurring nonenzymically and enzymically have been previously discussed (page 434), but in addition to these the quinones may undergo a variety of changes following administration to an animal or introduction into cell suspensions. The benzo- and naphthoquinones will be taken up separately here because their metabolisms generally differ. Polyphenols are conjugated with various groups in the liver and are partly excreted in these forms; the fate of the quinones is often similar since they can be reduced in the tissues. Thus administration of *p*-benzohydroquinone to dogs leads to the appearance
of sulfate conjugates in the urine (Ellinger, 1923; Speranskaya-Stepanova, 1940), whereas 3,5-dimethoxytoluquinone is conjugated mainly with glucuronate in the rabbit (Glock et al., 1945). Toluquinone is conjugated primarily with amino acids. What fraction of the benzoquinones is metabolized more drastically, e.g., by substitution of new groups on the ring or cleavage of the ring, is not known, although fungi can degrade p-benzo-hydroquinone to keto acids (Friedrich, 1956). The naphthoquinones appear to be reasonably stable in the body, as far as we know, since most of that administered appears eventually in the urine, but only menadione has been extensively studied. With respect to conjugation, the naphthoquinones do not differ markedly from the benzoquinones, the urinary products being sulfate esters and glucuronides (Richert, 1951; Jacques et al., 1954; Marrian and Maxwell, 1956; Bray and Garrett, 1961), although there is evidence for a phosphate also in the chromatographic separation used by Jacques. It may be noted that there is nothing from the excretory data to support reactions of the quinones with thiols, although quite possibly they have simply not been detected. Exactly what happens to the quinones which do react with the thiols in the blood and tissues is not known, and there is no doubt that such reactions occur (Canady and Roe, 1956).

The fate and metabolism of the phosphorylated quinols are especially important in understanding how they exert their antimitotic effects. Administration of menadiol-diP32 to rats and tracing of it and P32 in the tissues led Neukomm et al. (1953) to conclude that menadiol-diP penetrates into cells — that the phosphate groups indeed facilitate the penetration — and that some of the menadiol-diP is fixed and the rest is dephosphorylated. There may even be some exchange between menadiol-diP32 and P32. It was also thought that tissues with the most rapid division rate incorporate the menadiol-diP most readily. Morrison and Crowley (1952) also used menadiol-diP32 but concluded that the phosphate groups are rapidly split off and the radioactivity is distributed as P32. Menadiol-diP is dephosphorylated in human plasma by alkaline phosphatase but at physiological pH the rate is fairly slow, so that it can be transferred throughout the body without much hydrolysis (Ramasarma et al., 1959). Marrian and Maxwell (1956) preferred to use menadiol-diP-2-C14H3 because of the stability of the isotopic position, and 90 min after administration to rats could recover around 70% of the activity (33% in the urine, 5.5% free in the blood and tissues, 1% in the respiratory CO2, and 31% fixed in some form in the tissues). There is no particular pattern of uptake by the tissues of tumor-bearing rats, only the kidney showing a significantly more elevated level, and the concentrations are uniformly low, with differential absorption ratios in the range 0.1–0.4 for most tissues. Thus very little is metabolized to release the 2-methyl group as C14O2, but what happens to the 25–30% which is unaccounted for? These results do not bear directly on the problem
of the fate of menadiol-diP. The low tissue levels might be taken as evidence for the poor penetration of the substance, but Solvonuk et al. (1952) reported that menadione-2-C\textsuperscript{14}H\textsubscript{3} does not readily enter the tissues. Although this may be due in part to serum binding, it makes it impossible to interpret the data of Marrian and Maxwell in terms of the metabolism or distribution of the phosphate.

**NATURALLY OCCURRING QUINONES**

Several groups of naturally occurring quinones, such as the ubiquinones, the members of the vitamin K series, and certain antibiotics, have been discussed, but a treatment of the biochemistry and pharmacology of the quinones would not be satisfactory without at least brief mention of the sources of some of the quinones used commonly in metabolic studies, and some speculation as to their role, if any, in plants and animals. We can restrict our comments to certain aspects of the subject inasmuch as excellent reviews of the naturally occurring quinones are available (Hoffmann-Ostenhof, 1950; Venkataraman, 1957; Thomson, 1957, 1962). Nearly 200 different quinones have been isolated, the bulk of these coming from angiosperms and fungi. The only animals containing quinones other than the ubiquitous coenzymes are certain insects and marine invertebrates, especially the echinoderms (although occasionally a quinone is detected in other phyla, e.g., xyloquinone in a South American spider). Some beetles when stimulated emit a mixture of methyl- and ethyl-\(p\)-benzoquinones as a defense mechanism, and possibly other quinones may serve as sex attractants in insects, but the function of the echinochromes and spinachromes in the sea urchins is still debatable. Poisoning from the ingestion of certain plants has been attributed to their content of quinones. \(p\)-Benzoquinone is responsible for the poisoning of farm animals eating various species of *Xanthium* (cocklebur) (Kuzel and Miller, 1950). Other plants known in history as having medicinal properties often contain quinones but seldom has relation between the pharmacological actions and the quinones been established. We have noted that *Arctostaphylos uva-ursi* (bearberry) has been used for many years as a urinary antiseptic and for other purposes, the active principle presumably being arbutin, a glucoside of \(p\)-benzoquinone (page 543). Another plant which has a long medical history in the Orient is *Plumbago* (leadworts), which contains plumbagin (5-hydroxyxmenadione), although it is doubtful if the actions for which it has been mainly used are dependent on this quinone. However, there is no question about the clinical efficacy of the various anthraquinone cathartics of the emodin type obtained from rhubarb, cascara, senna, and other plants. One cannot help but wonder if the use of the African *Diospyros tricolor* (related to the ebony tree and the persimmon) in leprosy is related to its content of diosquinone (8-hydroxy-1,2-naphthoquinone).
Many of the quinones mentioned above in metabolic or antimitotic studies occur naturally. *p*-Benzoquinone, toluquinone, and the xyloquinones occur in either plants or insects, while the methoxy- and dimethoxy-*p*-quinones apparently are found in wheat germ. Lawson (2-hydroxy-1,4-naphthoquinone) is the dye in henna (*Lawsonia alba*), lapachol is a yellow pigment in the wood of *Tecoma* (greenheart) and other trees, lomatiol is a dye obtained from the seeds and bark of species of *Lomatia*, juglone is found in various parts of walnut trees, and phthiocol was originally derived from *Mycobacterium tuberculosis*. All of these and other quinones are occasionally quite potent inhibitors of metabolism or mitosis. Do they function in any way in the control of metabolism or growth in the microorganisms on plants? Almost nothing is known about this interesting aspect of the problem, but it has been suggested that they may regulate the rate of mitosis in growing tips or participate in oxidation-reduction reactions, for neither of which there is much evidence. In fact, it has been pointed out that certain fungal mutants which do not form a quinone normally present manage to proliferate and survive without difficulty. One might also raise the question as to how tissues which contain inhibitory quinones resist their actions. An important factor here is probably the fact that these quinones occur within the plant cells to a large extent as glycosides and hence are inactive. However, the quinones sometimes occur in a free form and apparently in rather high concentrations; e. g., lawson may be around 1% in henna leaves, lomatool 3.5% in the fruit and 12% in the seeds of *Lomatia*, and juglone nearly 8% in the walnut catkin buds. They may not be distributed uniformly and possibly in some instances are mainly outside the cells.

**AMINOPHENOLS AND PHENYLENEDIAMINES**

The ability to form quinonoid structures is also possessed by compounds in which the phenolic hydroxy groups of *o*- and *p*-benzohydroquinones are replaced by amino groups, and there is some evidence that these substances often exert metabolic actions similar to those of the quinones and hydroquinones. Early interest in *p*-aminophenol stemmed from the fact that it is a major metabolite of certain antipyretics and analgetics, such as acetonilide and acetophenetidin, and was thought by some to be responsible for the pharmacological effects of these drugs, while *p*-phenylenediamine attracted attention because of its toxic actions when used as a hair dye and industrially in the dyeing of fur and feathers and the vulcanization of rubber. Battelli and Stern (1912) demonstrated that *p*-phenylenediamine is readily oxidized by various mammalian tissue preparations, and it has been used by biochemists since that time as a hydrogen donor to the cytochrome system and as a cytological stain for oxidases. More recently there
has been a good deal of interest in \( p \)-phenylenediamine and its \( N \)-alkyl derivatives, since they arise in the metabolic degradation of carcinogens such as butter yellow (\( p \)-dimethylaminoazobenzene) and may be the active agents (Keusler et al., 1942b). The following discussion does not aim to be complete but only to compare chemically and biochemically these amines and their quinoneimines with the hydroquinones and quinones.

**Chemical Properties**

These amines can undergo oxidation-reduction reactions analogous to the hydroquinone-quinone reaction:

\[
\begin{align*}
\text{OH} & \quad \text{O} \\
\text{NH}_2 & \quad \text{NH} \\
\text{NH}_2 & \quad \text{NH} \\
\end{align*}
\]

\( p \)-Aminophenol \quad \text{p-Quinoneimine}

\[
\begin{align*}
\text{NH}_2 & \quad \text{NH} \\
\text{NH}_2 & \quad \text{NH} \\
\end{align*}
\]

\( p \)-Phenylenediamine \quad \text{p-Quinonediimine}

The redox potentials are somewhat higher than for \( p \)-benzoquinone (Fieser, 1930a). Semiquinonediimines are also formed and are often more stable than the semiquinones. Whereas the semiquinones are more stable at alkaline pH, however, the semiquinonediimine free radicals become more stable as the pH is lowered, since this favors the cationic form (Michaelis, 1935). \( p \)-Aminophenol and \( p \)-phenylenediamine in solution are oxidized by oxygen, and the rate increases with the pH (Bernheim and Bernheim, 1938). Indeed, the quinonoid forms used in metabolic experiments have usually been prepared by allowing solutions of the amines to stand at room temperature or \( 37^\circ \) for 30-60 min. The solutions become dark and this is taken as evidence for the oxidation. However, if this is done around neutrality, one will presumably not end up with much of the quinonoid forms, since they are quite unstable. The half-life of \( p \)-quinoneimine at pH 7.57 is 1.1 min and of \( p \)-quinonediimine 0.1 min (Fieser, 1930a). This
instability may be due to the 1,4 addition of an amino group, which may be followed by reoxidation to the quinonoid form and successive additions leading to polymerization. The \( pK_a \) of the amino group of \( p \)-aminophenol

\[
\begin{align*}
\text{NH} & \quad + \quad \text{H}_2\text{N}\text{–} & \quad \text{–NH}_2 \\
\text{NH} & \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad 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Inhibition of Enzymes

It is impossible to compare the inhibitory potencies of \( p \)-benzoquinone, \( p \)-quinoneimine, and \( p \)-quinonediimine from the data available, since the latter compounds have not been tested directly. Comparisons of the relative activities of the reduced forms are complicated by two factors: the different rates of oxidation to the quinonoid forms (which will depend on the pH), and the relative stabilities of the quinonoid forms. If one assumes that only the quinonoid form is inhibitory, the different degrees of inhibition observed may relate more to the pseudosteady-state concentrations of the quinonoid forms, rather than to their inherent inhibitory potencies. In other words, we never know the true concentration of the active form present.* The results obtained by Potter (1942) on urease at pH 5, where oxidation proceeds slowly, are thus useless for comparative purposes, and show only that the quinonoid forms are active (see accompanying tabulation). Oxida-

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (mM)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p )-Benzoquinone</td>
<td>0.0033</td>
<td>87</td>
</tr>
<tr>
<td>( p )-Benzyohydroquinone</td>
<td>1</td>
<td>75</td>
</tr>
<tr>
<td>( p )-Aminophenol</td>
<td>1</td>
<td>84</td>
</tr>
<tr>
<td>( p )-Phenylenediamine</td>
<td>1</td>
<td>45</td>
</tr>
<tr>
<td>( N )-Methyl-( p )-phenylenediamine</td>
<td>1</td>
<td>66</td>
</tr>
<tr>
<td>( N,N )-Dimethyl-( p )-phenylenediamine</td>
<td>1</td>
<td>64</td>
</tr>
</tbody>
</table>

* If the cytochromes are present in the enzyme preparation, the relative rates at which the reduced forms are oxidized will to some extent determine the inhibitions observed. Horio (1958) has shown that \( p \)-benzohydroquinone and \( p \)-phenylenediamine are oxidized at equivalent rates by cytochrome_\( eg \) and cytochrome oxidase from *Pseudomonas aeruginosa*, but that \( p \)-aminophenol is oxidized faster by cytochrome_\( eg \), and slower by the oxidase.
was sufficient time for complete oxidation under the conditions. The identical inhibitions given by \( p \)-benzohydroquinone, \( p \)-phenylenediamine, and the \( N \)-methylated derivates might point to equivalent inhibitory potencies of the quinonoid forms, but it may be that the inhibitions were limited by depletion of inhibitor. In any event, there is no evidence from any of this work of marked differences in the potencies of these compounds.

Other reported comparisons are even less reliable for a variety of reasons. Kensler et al. (1942 b) used a yeast apozyme preparation supplemented with NAD and utilizing fructose-1,6-diP, so the exact site of action is not known although it is likely to be the 3-phosphoglyceraldehyde dehydrogenase (see accompanying tabulation). Results of this type lend some support to the concept that the semiquinone form is active. Cohen et al. (1942) examined heart transaminase at pH 7 and incubated the enzyme with the inhibitors for 15 min (see accompanying tabulation). This enzyme appears to be much less readily inhibited by the quinonediimines than by the quinone. They doubted if reaction with SH groups is involved here.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (mM)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p )-Benzoquinone</td>
<td>0.01</td>
<td>65</td>
</tr>
<tr>
<td>( p )-Benzohydroquinone</td>
<td>0.01</td>
<td>65</td>
</tr>
<tr>
<td>( p )-Aminophenol</td>
<td>0.033</td>
<td>43</td>
</tr>
<tr>
<td>( p )-Phenylenediamine</td>
<td>0.01</td>
<td>65</td>
</tr>
<tr>
<td>( o )-Phenylenediamine</td>
<td>0.033</td>
<td>37</td>
</tr>
<tr>
<td>( N ),( N )-Methyl-( p )-phenylenediamine</td>
<td>0.01</td>
<td>65</td>
</tr>
<tr>
<td>( N ),( N )-Dimethyl-( p )-phenylenediamine</td>
<td>0.01</td>
<td>71</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (mM)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p )-Benzohydroquinone</td>
<td>0.22</td>
<td>0</td>
</tr>
<tr>
<td>( p )-Aminophenol</td>
<td>0.23</td>
<td>6</td>
</tr>
<tr>
<td>( p )-Phenylenediamine</td>
<td>0.23</td>
<td>75</td>
</tr>
<tr>
<td>( N ),( N )-Dimethyl-( p )-phenylenediamine</td>
<td>0.18</td>
<td>100</td>
</tr>
</tbody>
</table>
since iodoacetate does not inhibit. Süllmann (1943) found lipoxidase to be inhibited 35% by \( p \)-benzohydroquinone and 76% by \( p \)-aminophenol at 10 mM, pH 6.3, and 10-min incubation, but we know nothing of the mech-

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (mM)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p )-Benzoquinone</td>
<td>0.35</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>66</td>
</tr>
<tr>
<td>( p )-Phenylenediamine</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>( N )-Methyl-( p )-phenylenediamine</td>
<td>14</td>
<td>55</td>
</tr>
<tr>
<td>( N,N )-Dimethyl-( p )-phenylenediamine</td>
<td>14</td>
<td>43</td>
</tr>
</tbody>
</table>

anism of inhibition of this enzyme. Boyland and Gallico (1952) reported that \( N,N \)-diethyl-\( p \)-phenylenediamine is around 13 times more inhibitory than \( p \)-benzohydroquinone to catalase, but this may be related to the differences in redox potential rather than to the SH reactivity of the quinonoid forms.

It is probably true that the semiquinonediimines are usually much more stable than the quinonediimines, and thus are likely to play an important role in the inhibition of enzymes, as has been emphasized by Kensler et al. (1942 a) from studies of yeast pyruvate decarboxylase, which is inhibited well by the \( N \)-alkylated \( p \)-phenylenediamines whose free radicals are the most stable. For example, \( N,N,N',N' \)-tetramethyl-\( p \)-phenylenediamine inhibits 96% at 0.5 mM, whereas \( N,N,N',N' \)-tetramethyl-\( o \)-phenylenediamine inhibits only 10% and \( p \)-aminophenol only 15%. Elson and Hoch-Ligeti (1946) also studied the effects of phenylenediamines on urease, but allowed the solutions to stand 1–7 days in order to form the oxidation products, so that little information can be obtained from their results, except confirmation of the fact that \( m \)-phenylenediamine is a weaker inhibitor than the \textit{para} and \textit{ortho} isomers and shows no increased activity on standing. Their results on liver succinate oxidase are more interpretable because of the rapid oxidation of the compounds through the cytochromes, but differ from those of Potter in that the \( N \)-methylated derivatives appear to be much more potent than \( p \)-phenylenediamine. In fact, the inhibitions obtained were roughly one tenth those recorded by Potter, which is odd because the experiments appear to have been conducted identically. Differences in homogenate concentration may have been responsible, but if they were it is evident that one cannot interpret any of this work quanti-
tatively.\textsuperscript{*} \textsuperscript{\textdagger} \textsuperscript{N,N',N'\textdagger}-Tetramethyl-\textsuperscript{p}-phenylenediamine is often a rather potent enzyme inhibitor. In addition to the results above, it has been found to inhibit several enzymes at concentrations around 0.01 m\textit{M}, e.g., the nitrate reductase of the halophilic \textit{Vibrio costicolus} (Robinson, 1954). It can also serve as an electron donor and has been used to study terminal phosphorylations in the respiratory sequence (Howland, 1963 a). P:O ratios around 1.0–1.3 were obtained with rat liver mitochondria. On the other hand, it had been shown by Park \textit{et al.} (1957) that liver mitochondria oxidize this substance to a deep purple free radical, which at 0.2 m\textit{M} uncouples phosphorylation associated with the oxidation of \textit{\beta}-hydroxybutyrate, dropping the P:O ratio from 2.4 to 0.5. The uncoupling is antagonized by Mg\textsuperscript{++}, EDTA, and glutathione, and in each case there is a visible reduction in the purple color. There is little or no evidence to suggest that the actions of tetramethyl-\textsuperscript{p}-phenylenediamine are in any way related to reactions with SH groups.

There has been a good deal of work and argument relative to the effects of \textsuperscript{p}-aminophenol on xanthine oxidase, since Bernheim \textit{et al.} (1937) claimed that no other dehydrogenases in liver suspensions are inhibited at concentrations of \textsuperscript{p}-aminophenol which inhibit almost completely. Neither \textsuperscript{p}-benzoquinone nor \textsuperscript{o}-aminophenol was found to possess this selectivity of action. Using oxidized \textsuperscript{p}-aminophenol (i.e., solutions allowed to stand for a short while), Berheim and Berheim (1938) found 50\% inhibition at a concentration of 0.013 m\textit{M} (this is the total concentration and we do not know the concentration of \textsuperscript{p}-quinoneimine). They emphasized that this is a selective and somewhat unique type of dehydrogenase inhibition. Consistent results could not be obtained with milk xanthine oxidase and it was shown that milk contains some substance which inactivates the quinoneimine. Ball (1939) found that it requires 7 m\textit{M} \textsuperscript{p}-aminophenol to inhibit milk xanthine oxidase 77\%. Richert \textit{et al.} (1949) in general confirmed the results of the Bernheims and also found that the inhibition does not occur in the presence of substrate, indicating that \textsuperscript{p}-quinoneimine (or some other oxidation product) reacts readily at the substrate site. However, Doisy \textit{et al.} (1955), using a more purified preparation of rat liver xanthine oxidase and chicken liver xanthine dehydrogenase, observed very little inhibition by \textsuperscript{p}-quinoneimine at 0.2 m\textit{M}; although this was not explained, it might mean that the actual inhibitor is a complex of \textsuperscript{p}-quinoneimine with some liver component, or some component altered by the \textsuperscript{p}-quinone-

\* It would seem reasonable in experiments with reactive and tightly bound inhibitors acting on enzymes in homogenates to pay more attention to the kinetics of the inhibition. Likewise in situations in which the active inhibitor is being formed from a precursor it would be advisable to study the development of the inhibition with time. More significant results would also be obtained if some effort toward purifying enzyme preparations was made.
imine. Gray and Felsher (1945), on the basis of the xanthine oxidase inhibition, injected p-aminophenol intravenously in a patient with urate gout, administering 0.5–4 g daily, and observed no changes in either blood or urine levels of urate, thus concluding that no inhibition occurs in vivo, possibly due to the p-aminophenol remaining in the reduced form. Although Bernheim et al. (1937) found no other dehydrogenases susceptible to p-aminophenol, the endogenous respiration of rat liver suspensions is fairly well inhibited (see accompanying tabulation), so that some enzyme in-

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (mM)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>p-Benzquinone</td>
<td>0.44</td>
<td>25</td>
</tr>
<tr>
<td>p-Benzhydroquinone</td>
<td>0.44</td>
<td>32</td>
</tr>
<tr>
<td>p-Aminophenol</td>
<td>0.11</td>
<td>56</td>
</tr>
<tr>
<td>o-Aminophenol</td>
<td>0.11</td>
<td>46</td>
</tr>
<tr>
<td>p-Phenylenediamine</td>
<td>0.11</td>
<td>36</td>
</tr>
<tr>
<td>o-Phenylenediamine</td>
<td>0.11</td>
<td>51</td>
</tr>
<tr>
<td>m-Phenylenediamine</td>
<td>0.11</td>
<td>16</td>
</tr>
</tbody>
</table>

volved in this uptake of oxygen must be sensitive. Unfortunately, one does not know a great deal about the nature of such endogenous respiration.

**Effects on Proliferation and Growth**

Alcalay (1947 a) tested 18 naphthoquinones for bacteriostatic activity (see Table 5-9), and found the most potent substance to be 2-amino-1,4-napthoquinone-imine, bacteriostatic concentrations being in the range 0.01–0.04 mM and even *E. coli* being readily inhibited. It was found to be much more toxic to mice; the maximal tolerated dose of 5 mg/kg causing convulsions; the naphthoquinones were tolerated at doses of 35–400 mg/kg (Alcalay, 1947 b). However, the naphthoquinoneimines have not been thoroughly studied and it is impossible to assign a mechanism for this growth inhibition. *N,N,N',N'-Tetramethyl-p-phenylenediamine* does not appear to be virucidal or virustatic (Czekalowski, 1952). *N,N-Dimethyl-p-phenylenediamine* at 0.22 mM causes a marked increase in the respiration of sea urchin eggs, which is maximal at 2.2 mM, but in spite of this the development proceeds normally (Runnström, 1932). This indicates that it serves here as an oxidizable substrate and does not interfere with important metabolic processes in the eggs (indeed such diamines are oxidized quite rapidly at the pH of sea water in the absence of living material).
When \( p \)-phenylenediamine is added to melanoma extracts there is a rapid increase in the oxygen uptake, and this is unique because the extra respiration is not inhibited by cyanide (Riley, 1958). It was found that this arises from a nonenzymic reaction between \( p \)-phenylenediamine and dopa. Mice bearing melanomas are less susceptible than normal mice to the toxic effects of \( p \)-phenylenediamine, while they are more susceptible to \( o \)-phenylenediamine. These relationships led to the testing of the diamines for cinostatic activity (Riley, 1959). \( o \)-Phenylenediamine was found to be more effective and less toxic than \( p \)-phenylenediamine, and could suppress the in vivo growth of mouse and hamster melanomas and Ehrlich solid carcinomas. Complete inhibition of the latter tumor could be achieved and occasionally complete remission of established tumors. It is not known if the reaction with catecholamines is involved in the growth inhibition. The phenylenediamines were found to inhibit the respiration of Ehrlich ascites cells but to have little or no effect on the glycolysis, and it was concluded that the NAD-linked enzymes are not the sites of attack, as had been suggested by Kensler et al. (1942 b). However, since the data and conditions were not presented, it is impossible to judge the validity of this conclusion.

**Effects on Tissues and in Whole Animals**

The pharmacology of the phenylenediamines and their methyl and dimethyl derivatives was well studied by Hanzlik (1923 a, b). The toxic and fatal doses seem to be of the same magnitude as for \( p \)-benzohydroquinone (see Table 5-5), namely, around 100–200 mg/kg subcutaneously, although the methylated derivatives are somewhat more toxic. The effects on the heart and circulation are complex and presumably related to direct actions on the vessels and myocardium since they are independent of innervation. The heart is not very sensitive to the phenylenediamines and stops in diastole at high concentrations or doses. No mention was made of contracture in either the heart or skeletal muscles. There is surprisingly little difference in the effects of the \textit{para} and \textit{meta} isomers, which may indicate that the quinonoid form is not involved in the toxicity. Tainter and James (1929) found that \( o \)-phenylenediamine is not only weaker pharmacologically than the \textit{para} isomer, but produces a different pattern of effects. It is difficult in any of this work to compare the phenylenediamines with the quinones because they were never examined under comparable conditions.

The phenylenediamines are irritants and vesicants (Hanzlik, 1923 a); they share this action with \( p \)-benzoquinone and other penetrable SH reagents, but whether this is due to inhibition of pyruvate oxidation is not known. \( p \)-Phenylenediamine and \( p \)-benzoquinone act very similarly on the conjunctiva, producing inflammation and edema (Estable, 1948).
However, many different skin reactions occur following exposure to the phenylenediamines; some are probably attributable to a sensitization, which Mayer (1948) related to the ability of \( p \)-quinoneimine and \( p \)-quinonedimine to react with the SH and amino groups of skin proteins. He pointed out that 2,5-diamino-\( p \)-xylene, which can be oxidized to the quinonedimine but would not react readily with these protein groups, is not a sensitizer. A correlation between epithelial sensitization and carcinogenicity was suggested.

An interesting reaction to \( p \)-phenylenediamine is the rapid development of facial and neck edema (Hanzlik, 1923 a). \( m \)-Phenylenediamine does not produce this although it causes some pulmonary edema. \( o \)-Phenylenediamine is even less potent (Tainter et al., 1929). Edematous swelling of the neck muscles and skin, the tongue, and the larynx reaches a maximum in around 2 hr after administration, but can be seen as early as 15 min after injection (Fuchs, 1963). The tongue capillaries show separation and reduction of the endothelial cells, osmiophilic thickening of the ground substance, and sections in which the flow no longer occurs. Numerous small vacuoles appear in the capillary endothelial cell cytoplasm and pathological changes are observable in the mitochondria and nuclei. Death of the animal may result from the asphyxia brought about by the swelling. Such a remarkable selectivity of action on certain capillary beds (assuming that the direct action is indeed on the capillaries) is unique among enzyme inhibitors or SH reagents, and it would be most interesting to determine whether this action has a metabolic basis. It is not evident with the benzoquinones, so that it is not justifiable at the present time to relate the edema to a quinonoid structure.
Compounds of arsenic were used in medicine two to three thousand years ago in the Orient and sporadic therapeutic applications have continued through the ages. Meanwhile, arsenic also gained the reputation of a dangerous poison, and during the fifteenth to seventeenth centuries was the principal ingredient of most of the homicidal concoctions of the famous poisoners. Much effort has been expended over the past century to elucidate the mechanisms by which the arsenicals produce their pharmacological and toxicological effects. A detailed chronological account of these developments is unnecessary at this time and would lead us far afield. Instead we shall be content with a few observations signifying the highlights in arsenical research, and then discuss in more detail the history of certain aspects in their appropriate sections. Excellent discussions of the early work may be found in the reviews of Voegtlin et al. (1923), Heftter and Keeser (1927), Keeser (1937), Work and Work (1948), Peters (1955), Doak and Freedman (1960), and Buchanan (1962), and in various textbooks of pharmacology and toxicology.

The primary stimuli for arsenical research have come from (1) the long history of arsenic as a homicidal poison, (2) the industrial use of the arsenicals and the resultant exposures to them, (3) the clinical application of the arsenicals in protozoal and neoplastic diseases, and (4) the use of arsenicals in chemical warfare. Arsenical poisoning has been brought about in a variety of ways: wallpaper pigments, cattle dips, insecticides, herbicides, and many different types of industrial exposure have all contributed; in addition, toxic reactions during the clinical use of the arsenicals have unfortunately been rather common. Arsenicals have been used at one time or another in almost every type of disease. Inorganic arsenite has its only valid present use in chronic myelocytic leukemia, but has been widely used as a tonic and in a variety of dermatoses (e.g., dermatitis herpetiformis) on the basis of its supposed ability to increase the blood flow through certain tissues, especially the skin, and in intractable bronchospasm. Arsenite was known to have some effect in trypanosomiasis but its toxicity precluded its use. A major event in the arsenical story
was the discovery by Thomas and Breinl in 1905 of the value of atoxyl (sodium arsenilate) in murine trypanosomiasis; in the same year Koch showed it to be effective in human trypanosomiasis. Atoxyl had been known chemically since 1863 and had been shown to be inactive in vitro by Ehrlich in 1903. These observations initiated the impressive series of organic arsenicals used for the treatment of syphilis (arsphenamine in 1910 and oxophenarsine in 1932), trypanosomiasis (tryparsamide in 1919), and amebiasis (acetarsone in 1924 and carbarsone in 1931), not to mention many of the more recent derivatives. These compounds are often more interesting metabolic and enzyme inhibitors than the inorganic arsenicals. The importance of the trypanosome work can scarcely be overestimated, since it and the concepts formulated by Ehrlich and his collaborators have formed the basis for all the specific chemotherapy since that time, and were the first attempts to visualize the lethal actions of chemicals in terms of specific receptor groups within the cell. Ehrlich (1909) demonstrated that the pentavalent arsenicals must be reduced to the trivalent forms to be active, that they do not act by release of inorganic arsenite, and that the actions can best be explained by assuming vital arsenerceptors.*

The early work was much concerned with the comparison of the effects of arsenite and arsenate, and the possibility that the tri- and pentavalent forms are interconverted in the tissues. Although earlier attempts to settle these problems were made, the preparations were often impure, and so we must attribute to Ringer and Murrell (1878) the first clear demonstration that arsenite is more toxic than arsenate when injected into frogs, and that arsenite acts more rapidly. Loew (1887) in general confirmed these results in plants and the lower animals, but claimed that some of the fungi and higher animals are equally sensitive or insensitive to arsenite and arsenate. Kionka (1911) felt that the issue was not yet settled and performed various experiments with opalinids, frogs, and rabbits which purported to show the greater activity of arsenite, but Joachimoglu (1915) criticized these results on the basis that data from whole animals are not able to establish the relative toxicities, and proceeded to show that isolated tissues are more sensitive to arsenite than to arsenate. It was soon realized that the pentavalent organic arsenicals are not directly inhibitory and are reduced in the body to the active forms. All recent work has fully established that arsenite and arsenate act very differently, but the possibility of their interconversion in the systems studied must always be borne in mind. We shall not discuss the actions of arsenate in

* "Ich bin, um es vorweg zu nehmen zu der Anschauung gelangt, dass in Protoplasma der Trypanosomen-Zellen gewisse Gruppierungen vorhanden sind, die imstande sind, sich mit dem dreiwertigen Arsenrest zu verbinden, und die ich deshalb in Anlehnung an die Terminologie der Immunitatslehre als 'Arsenceptoren' bezeichne" (Ehrlich, 1909).
this chapter, since it is not an SH reagent and interferes with phosphorylations instead, but the pentavalent organic arsenicals will be included because they produce their effects after reduction. A phenomenon observed as early as 1896 is that certain organisms become increasingly resistant to the arsenicals during continuous exposure. Because of the practical aspects of this in chemotherapy, it has been intensively studied and has provided much information relevant to the basic actions of the arsenicals. Any complete theory of arsenical action must include some explanation of resistance.

The toxic effects of arsenite on a variety of microorganisms, plants, and invertebrates have been studied since 1850, and the concept was evolved that it is a so-called protoplasmic poison, exerting a nonspecific protein-coagulating action, a theory no longer valid. The idea that arsenicals produce their effects by interfering in some manner with metabolism is also very old and a good deal of work was done in the nineteenth century to demonstrate this, but the results led to no definite conclusion. The difficulty was mainly in finding the right enzymes or metabolic systems to be tested, and the many negative results discouraged some workers into believing that a metabolic explanation for the mechanism is not valid. The earliest metabolic work which has come to my attention is that of Basilius Savitsch (1854), working at Dorpat under Buchheim’s direction. He reported that yeast fermentation is depressed by arsenite, although the action is rather weak and slow to develop. Johannsohn (1874) continued this work at Dorpat and found fermentation to be reduced 16% by 5.5 mM arsenite over a period of 24 hr; an inhibition of 26% by 9.2 mM arsenite was also observed. The growth of yeast is definitely inhibited at these concentrations. On the other hand, Schulz (1888) obtained a fairly potent inhibition of yeast fermentation, 0.25 mM arsenite depressing in around 30%, and 0.7 mM around 65%. The reason for this discrepancy in potency is not immediately apparent. These observations on yeast did not seem to influence significantly the concepts of arsenical action, and similar experiments to answer similar problems were being done many years later.

Pharmacological studies in the meantime were being made by Sklarek (1866), who recorded a primary central action leading to reflex depression and motor paralysis, and by Ringer and Murrell (1878), who confirmed the central action but also observed the more rapid occurrence of rigor mortis in poisoned muscles and hence a direct effect on skeletal muscle. Several reports pointed to some metabolic interference in whole animals, but much argument ensued as to whether these effects, are primary or secondary. For example, Cunze (1866) concluded that the decrease in body temperature caused in rabbits by arsenite indicates a metabolic depression, but Von Boeck (1871) correctly surmised that such evidence is not satisfactory. On the other hand, Von Boeck’s demonstration of an effect on nitrogen
balance in the dog is also not proof of a direct action on metabolism. Meyer (1881) found that the blood CO$_2$ is reduced in rabbits poisoned by arsenite and obtained evidence that some acid, probably lactic acid, appears in the blood, indicating a disturbance in carbohydrate metabolism, while Araki (1893) showed that lactate appears in the urine under such conditions. The conversion of arsenite to arsenate in some tissues was observed by Spitzer (1898) and he investigated tissue respiration with the idea that arsenite might serve as an oxygen acceptor (i.e., as an oxidizable substrate), but found only depression instead of stimulation.

It is often difficult to understand why a particular observation, intrinsically of little significance or very similar to the results of others, is occasionally responsible for stimulating further important work. In the present field it was the demonstration by Onaka (1911) at Heidelberg that arsenite in low concentration (0.023 mM) inhibits the respiration of goose erythrocytes. Such results must come at the right time and place to be influential. It was at this time and place that Warburg was working on the inhibitory effects of cyanide and carbon monoxide; thus Warburg became interested in arsenite as a respiratory inhibitor, although for some years under the mistaken impression that it acts like cyanide on the iron-containing enzymes involved in oxygen activation, an error shared by several others during this period. Battelli and Stern (1911) at the same time made the observation that, although arsenite acts on respiration about as well as cyanide, it depresses succinate oxidation by muscle minces much less readily, around 5 mM being required for definite inhibition, results which should have given a clue to the basic difference in the sites of action of these inhibitors. Much later, Dresel (1926) was still assuming that arsenite behaves like cyanide and carbon monoxide in depressing respiration, and thought it would be a more useful inhibitor for this purpose because it is not volatile. Szent-Györgyi (1930) eventually showed that arsenite has virtually no effect, even at concentrations as high as 10 mM, on cytochrome oxidase using p-phenylenediamine as the substrate. A new approach to the action of the arsenicals was needed and this was provided by the study of Voegtlin et al. (1923), in which it was shown that various thiols can antagonize the effects of oxophenarsine on trypanosomal motility. In conjunction with the recently proposed general role of glutathione and SH groups in cellular metabolism, this led immediately to the concept that the arsenicals might react with thiols in the cells and interrupt oxidative processes. Further work by Voegtlin and his colleagues, and by others, has established this mechanism of inhibition, and there has been a steady increase in confidence that this is indeed the primary site of attack.

The next important advance was made by Krebs (1933 a, b) in showing that arsenite causes the marked accumulation of pyruvate and other keto acids during the metabolism of amino acids in kidney preparations.
It was evident that the oxidation of keto acids is rather specifically blocked by the arsenicals and this has been substantiated in many tissues and under a variety of conditions, so that today the keto acid oxidases are generally considered to be the key sites for the actions of the arsenicals. Many enzymes had been tested since 1870 but few had been found to be clearly sensitive to arsenicals, but the keto acid oxidases had not been available for investigation. World War II initiated intensive study of the arsenicals, particularly in the laboratory of Sir Rudolph Peters at Oxford, and it was demonstrated without question that pyruvate oxidase and the oxidation of pyruvate generally are extremely susceptible to arsenicals, this presumably being associated with the participation of the dithiol, lipoic acid, in the transfer of acyl groups and electron transport by these oxidases. One of the major purposes of this chapter is to provide evidence for this site of action and to examine critically the role of keto acid oxidase inhibition in the effects exerted by the arsenicals on cellular function and growth.

The arsenicals today are a commonly used group of SH reagents for the inhibition of enzymes and metabolic reactions. In some respects they may be as specific for SH groups as any inhibitor available; but we shall see that they differ markedly from most other SH reagents in the types of SH group reacted, and so can provide much information on the composition of active sites and the participation of certain thiols. The usefulness of the mercurials to the biochemist was increased when simple organic mercurials were introduced. Likewise, the organic arsenicals have occasionally been used to good advantage; however, they are perhaps not used as routinely, relative to arsenite, in metabolic work as they should be, and there has not been a concerted effort to investigate the relative activities of these compounds on enzyme systems. One must emphasize at the beginning that there is a paucity of good delineation of the over-all effects of the arsenicals on the metabolism of any organism or tissue, and essentially no studies bearing on the specificity of action in living tissue have been made. It is for this reason that one must make the regrettable statement that, despite what has been written to the contrary, we are not able confidently today to explain satisfactorily any action of the arsenicals in detail. It is an excellent field for the correlation of structure with action, and thorough studies of the effects of hundreds of compounds on microorganisms have been made, but these investigations have not been extended to enzymes or metabolism and thus are complicated by many factors, such as permeability.

**CHEMICAL PROPERTIES**

Discussion of the chemistry of the arsenicals is complicated by the fact that many different types of compound have been used in biological work. The nomenclature of the arsenicals has been confusing and lacks uniformity.
We shall, in general, adopt the terminology of Doak and Freedman (1960). (see accompanying tabulation). Most arsenicals can be named in different ways; e.g., phenyl-As=O can be called phenylarsine oxide, phenylarsenoxide, or arsenosobenzene. All the trivalent arsenicals are basically derivatives of arsine, AsH₃, and can be named on this basis. In tables of inhibition we shall frequently use abbreviations such as \( q^-\text{AsO} \) for phenylarsenoxide or \( q^-\text{AsO}_2 \) for phenylarsonic acid. These are not meant to indicate the state of the substance in solution but are used only for brevity. For example, \( q^-\text{As(OH)}_2 \) is the hydrated form of \( q^-\text{AsO} \), and \( q^-\text{AsO(OH)}_2 \) is the hydrated form of \( q^-\text{AsO}_2 \); in solution the hydrated form probably predominates. The names and structures of some of the commonly used *Radical name (prefix)  |  Generic name (suffix)  |  Type structure*  
---|---|---
**Trivalent**  
Arsino-  
-arsine  
Primary  
\( R^-\text{As}^-\text{H} \)  

Secondary  
\( R\backslash\text{As}^-\text{H} \)  

Tertiary  
\( R\backslash\text{As}^-\text{R}'' \)  

Arsenososo-  
-arsenoxide  
(arsine oxide)  
R=As=O  

—  
-arsonous acid  
R=As\( \text{OH} \)  

Arsinososo-  
-arsinous acid  
R\backslash\text{As}^-\text{OH}  

Arsecono-  
—  
R=As=As=R  

**Pentavalent**  
Arso-  
—  
R=As\( \text{O} \)  

Arsono-  
-arsonic acid  
R=As\( \text{OH} \)  

Arsinico-  
-arсинic acid  
R\backslash\text{As}^-\text{OH}
arsenicals are shown below, but again these do not necessarily represent the form in solution.

\[
\begin{align*}
\text{As}_2\text{O}_3 & \quad \text{AsO(OH)} & \quad \text{AsH}_3 & \quad \text{H}_3\text{C} \begin{array}{c} \text{As} \end{array} \begin{array}{c} \text{O} \end{array} \\
\text{Arsenic trioxide} & \quad \text{Arsenious acid} & \quad \text{Arsine} & \quad \text{H}_3\text{C} \begin{array}{c} \text{As} \end{array} \begin{array}{c} \text{OH} \end{array} \\
\begin{array}{c} \text{Cl} \end{array} \begin{array}{c} \text{CH}=\text{CH} \end{array} \begin{array}{c} \text{As} \end{array} \begin{array}{c} \text{Cl} \end{array} & \quad \begin{array}{c} \text{Cl} \end{array} \begin{array}{c} \text{CH}=\text{CH} \end{array} \begin{array}{c} \text{As}=\text{O} \end{array} & \quad \text{O} \begin{array}{c} \text{As} \end{array} & \quad \text{O} \begin{array}{c} \text{As} \end{array} \\
\text{Lewisite} & \quad \text{Lewsite oxide} & \quad \text{Phenylarsenoxide} & \quad \text{p}-\text{Amino-phenylarsenoxide} \\
\text{Oxophenarsine} & \quad \text{(Mapharsen)} & \quad \text{Dichlorophenarsine} & \quad \text{(Chlorarsen)} \\
\text{Phenylarsonic acid} & \quad \text{(atoxyl)} & \quad \text{Butarsen} & \quad \text{Melarsen oxide} \\
\text{Phenylarsonic acid} & \quad \text{Sodium arsanilate} & \quad \text{Carbarsone} & \quad \text{Arsacetin} \\
\text{Acetarsone} & \quad \text{Tryparsamide} & \quad \text{Arsphenamine} & \quad \text{Neoarsphenamine} \\
\text{Arsenophenylglycine} & \quad 2\text{Na}^+ & \quad \text{Neoarsphenamine} & \quad \text{Arrenophenylglycine}
\end{align*}
\]
Arsenious Acid and the Arsenites

Arsenious acid exists only in solution and is formed when arsenic trioxide is dissolved in water:

$$\text{As}_2\text{O}_3 + \text{H}_2\text{O} \rightleftharpoons 2 \text{AsO(OH)}$$

In much of the early metabolic work, arsenic trioxide was dissolved in acid solution and then neutralized with NaOH; this gives the same result as dissolving sodium metaarsenite (NaAsO$_2$), which is now generally used. Arsenic trioxide exists in at least three different forms and the aqueous solubilities differ: the vitreous form is the most soluble (3.7% at 37°), the octahedral form is less soluble (2.8%), and the monoclinic form is the least soluble (1.8%); saturated solutions are thus around 100 mM. The solubility varies with the pH but appreciable changes occur only far outside the biological pH range (Garrett et al., 1940). Arsenic trioxide dissolves very slowly in water; after 4 months of agitation in water, equilibrium is still not completely reached (Schnellbach and Rosin, 1929). The rate of solution is accelerated by alkalinization and increased temperature.

Since arsenite is commonly used in metabolic studies, it is important to inquire into the state of the substance in solution near neutrality. Inasmuch as the $pK_a$ is generally given as 9.22 (Sellers et al., 1964) [there is disagreement as to the exact value and a $pK_a$ of 9.62 was obtained by Garrett et al. (1940)], there would be relatively little of the anionic arsenite (AsO$_2^-$) at physiological pH’s. There is thus some question as to whether the term “arsenite” is strictly correct for designating the substance, but since it has been so commonly used it will be retained here. The conductance of solutions of arsensce acid is low, indicating the weak ionization. The monomeric form has been assigned different structures in solution, including metaarsenious acid (HAsO$_2$) and orthoarsenious acid (H$_3$AsO$_3$), which correspond exactly to metaphosphorous acid (HPO$_2$) and orthophosphorous acid (H$_3$PO$_3$); solid salts are either meta- or orthoarsenites (generally the former), but these give no information on the state in solution. We shall write arsensce acid as HAsO$_2$ or AsO(OH) for simplicity, but more highly hydrated forms must be considered as included. Solutions of arsenic trioxide are acid (around pH 5) and of arsenite salts are alkaline, and both must be adjusted to the correct pH before use. There is a second $pK_a$ near 13.5, probably corresponding to the formation of HAsO$_3^-$, but this is not biologically important. The protonation of arsensce acid to form As(OH)$_2^+$ (or AsO$^+$ or As$^{4+}$) occurs, but the $pK_a$ is around 1 so that it also need not be taken into account. The importance of polymerization has been debated for many years. In strongly alkaline solutions ($\geq$ pH 12), polymers exist in appreciable amounts: in 0.1 $M$ NaOH solution there may be about 25% as the dimer, HAs$_2$O$_4^-$, and 10% of the trimer, HAs$_3$O$_6^-$ (Gar-
rett et al., 1940), but it is doubtful if these polymers occur in sufficient concentrations near neutrality to be biologically important.

Aqueous solutions of arsénious acid are quite stable in air up to at least pH 10-11. However, if certain oxidants or catalysts are present, oxidation to arsenate may occur rapidly. Arsenite can be oxidized by Fe^{3+} and, if a further system is present whereby the Fe^{3+} can be reoxidized, arsenate will be formed when only catalytic amounts of Fe^{3+} occur (Wieland and Franke, 1928). Such reactions, furthermore, occur at neutrality. The redox system:

\[ \text{HAsO}_2 + 2 \text{H}_2\text{O} \rightleftharpoons \text{H}_3\text{AsO}_4 + 2 \text{H}^+ + 2\text{e}^- \]

has an \( E_0 = +0.559 \) v, so that \( E'_0 \) (pH = 7) would be near \(-0.27 \) v. Arsenite could thus be oxidized by a variety of substances present in tissues, and this will be discussed in greater detail in the section on the metabolism of arsenite (page 785).

A point often totally neglected, and occasionally of importance in work with enzymes and tissues, is the formation of poorly dissociated or relatively insoluble salts between arsenite and the alkaline earth or heavy metal ions. For example, if dilute CaCl\(_2\) solution is added to a solution of potassium arsenite, a precipitate of calcium arsenite, Ca(AsO\(_2\))\(_2\), appears, this being soluble to the extent of only 0.04% (around 1.8 mM). Thus the presence of these metal ions may decrease the free arsenite concentration and, conversely, arsenite in some cases may exert a secondary effect on the system studied by reducing the concentrations of enzymically or physiologically active cations, in a manner similar to that for malonate or fluoride.

It may be noted that the sodium and potassium arsenites available commercially are not always pure. They may contain mixtures of the meta- and orthoarsenites and, hence, for quantitative work the arsenite concentration should be determined. In addition, some preparations are 95% or less pure and are apt to contain active impurities. A pure sample of arsenic trioxide produced much less gastrointestinal damage than did the usual impure preparation (Harrison et al., 1958). Since the impure preparation contained 1.2% Sb\(_2\)O\(_3\) and small amounts of heavy metals it was suggested that the tissue damage might have been caused by these rather than the arsenical. Similar problems could, of course, arise in enzyme work.

**Organic Arsenicals**

The earliest therapeutic arsenicals were usually the pentavalent arsanic acids, but it was recognized by Ehrlich that reduction to the trivalent forms is probably necessary for activity, inasmuch as substances such as atoxyl are generally inactive *in vitro*, and become active upon reduction.
The arsenoxides (R—AsO) are enzyme inhibitors and biologically active because of the facility with which the arsenoxide group reacts with SH groups. The remainder of the molecule generally does not participate in the reaction, but serves to modify the properties of the molecule, especially with regard to the penetration into cells, the adsorption to proteins, and the solubility, and in addition modifies quantitatively the properties of the arsenoxide group with respect to its redox potential and ionization. Thus, in general, we may say that all these substances act ultimately by the same basic chemical reaction, but differ in the ability to reach the thiols or enzymes within living cells and in the rate at which mercaptide formation occurs.

As is the case with inorganic arsenite, there is no agreement as to the dominant form in aqueous solution, i.e., the extent of hydration. The arsenoxides are usually written as R—As=O, but it is possible that the hydrated R—As(OH)$_2$ forms are also important. If so, the $pK_a$'s must be quite high and the groups should exist mainly in the un-ionized state at physiological pH. Of course, acidic or basic substituent groups on the benzene ring may ionize to increase the solubility and provide compounds convenient to use in enzyme studies. The pentavalent phylarsonic acids, on the other hand, ionize readily, indicating that the substances exist in solution mainly in the R—AsO(OH)$_2$ form. Some ionization constants are given in Table 6-1. The preponderant form in the physiological pH range is thus the R—AsO$_3$H$^-$ anion, with variable and appreciable fractions in the R—AsO$_5^-$ form. These values may be compared with those for inorganic arsenic acid: $pK_{a1} = 2.30$, $pK_{a2} = 4.40$, and $pK_{a3} = 9.22$.

The phenylarsonous acids (or phenylarsenoxides) and the phenylarsonic acids form oxidation-reduction systems whose redox potentials are difficult to determine because of the slow attainment of equilibrium. Since it is assumed that most arsonates are active only after reduction, and since the question of the oxidation of the arsonous acids in tissue preparations is often pertinent, the rates of oxidation or reduction would be useful to know, as well as the thermodynamic equilibria when coupled with other redox systems. For example, it has been postulated that the activity of the phenylarsonates may be related to the speed at which they are reduced (Cohen et al., 1932). The rates of reduction are technically difficult to measure but the bimolecular rate constants for the oxidation of various phenylarsonous acids by cystine were determined (see accompanying tabulation). It may be mentioned that no correlation between these rates and the toxicity of the phenylarsonic acids was found, which is not surprising inasmuch as the rates of oxidation of the arsonous acids would bear no necessary relation to the rates of reduction of the corresponding arsanic acids. It is important to remember, however, that reduction can take place quite readily in biological systems, often by thiols with which
the trivalent forms will react. Both phenylarsonates and phenylarsenoxides are otherwise fairly stable in solution.

<table>
<thead>
<tr>
<th>Phenylarsonous acid</th>
<th>$k \times 10^5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p$-CH$_2$CONH$^-$</td>
<td>38.54</td>
</tr>
<tr>
<td>$p$-NH$_2$CO$^-$</td>
<td>34.45</td>
</tr>
<tr>
<td>Unsubstituted</td>
<td>32.57</td>
</tr>
<tr>
<td>$p$-NH$_2$COCH$_2$NH$^-$</td>
<td>30.45</td>
</tr>
<tr>
<td>$p$-CH$_3$O$^-$</td>
<td>30.05</td>
</tr>
<tr>
<td>$p$-Cl$^-$</td>
<td>25.04</td>
</tr>
<tr>
<td>$p$-HOOC$^-$</td>
<td>22.62</td>
</tr>
<tr>
<td>$p$-NH$_3$SO$_2$$^-$</td>
<td>22.43</td>
</tr>
<tr>
<td>3-NH$_2$-4-OH$^-$</td>
<td>19.9</td>
</tr>
<tr>
<td>$p$-OH$^-$</td>
<td>16.7</td>
</tr>
<tr>
<td>$o$-OH$^-$</td>
<td>2.82</td>
</tr>
</tbody>
</table>

**Table 6-1**

**Ionization Constants of Phenylarsonic Acids**

<table>
<thead>
<tr>
<th>Phenylarsonic acid</th>
<th>$pK_{a_1}$</th>
<th>$pK_{a_2}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unsubstituted</td>
<td>3.47</td>
<td>8.48</td>
<td>Pressman and Brown (1943)</td>
</tr>
<tr>
<td>$o$-Methyl</td>
<td>3.82</td>
<td>8.85</td>
<td></td>
</tr>
<tr>
<td>$m$-Methyl</td>
<td>3.82</td>
<td>8.82</td>
<td></td>
</tr>
<tr>
<td>$p$-Methyl</td>
<td>3.70</td>
<td>8.68</td>
<td></td>
</tr>
<tr>
<td>$p$-Hydroxy</td>
<td>3.89</td>
<td>10.05</td>
<td></td>
</tr>
<tr>
<td>$p$-Amino</td>
<td>2.0</td>
<td>8.92</td>
<td></td>
</tr>
<tr>
<td>$p$-Nitro</td>
<td>2.90</td>
<td>7.80</td>
<td></td>
</tr>
<tr>
<td>$p$-Chloro</td>
<td>3.33</td>
<td>8.25</td>
<td></td>
</tr>
<tr>
<td>Unsubstituted</td>
<td>3.59</td>
<td></td>
<td>Portnov (1948)</td>
</tr>
<tr>
<td>$p$-Methyl</td>
<td>3.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p$-Hydroxy</td>
<td>4.09</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p$-Amino</td>
<td>4.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$p$-Nitro</td>
<td>3.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arsanilic acid</td>
<td>3.97$^a$</td>
<td></td>
<td>Williamson (1959 a)</td>
</tr>
<tr>
<td>Arsacetin</td>
<td>3.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tryparsamide</td>
<td>4.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melarsen</td>
<td>4.97</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Values for the $pK_{a_1}$ were obtained by Williamson in 50% methanol and the values given were estimated to be those for aqueous solutions.
The phenylarsonates form fairly insoluble salts with various metal ions (Portnov, 1948), as do the inorganic arsenates and arsenites. Perhaps this should be borne in mind in biological work, although no thorough investigation of this has been made. The complexes formed between triphenylarsine oxide and ions such as Ni\(^{++}\) and Hg\(^{++}\) in organic solvents (e.g., ether or alcohol) have been studied by Phillips and Tyree (1961), but there is some question whether such complexes occur in aqueous media.

The arseno compounds (R—As=As—R), which include the previously used antiluetics, arsphenamine and neoarsphenamine, are unstable in solutions near neutrality in the presence of oxygen, and spontaneously split into the corresponding arsenoso compounds (R—As=O), which are biologically active (Eagle, 1939 a). There is thus very little reason for using these compounds either therapeutically or in enzyme studies. Substances such as tetraarsenoacetate (—OOC—CH\(_2\)—As=As—As=As—CH\(_2\)—COO—) are likewise active only after splitting to arsenite and arsenosoacetate (Barbour et al., 1925).

The preparations and chemical properties of the organic arsenicals are well covered in the book by Raiziss and Gavron (1923).

### Atomic Properties and Bond Characteristics of Arsenic

Crystallographic studies of arsine, arsениmethane, lewisite, arsenobenzene, phenylarsonic acid, the cacodyl halides, and various arsenicals (Nielsen, 1952; Shimada, 1960; Hedberg et al., 1961) have provided the mean bond lengths shown in the accompanying tabulation (the As—C distance seems to be nearer 1.90 \(\text{Å}\) in aromatic compounds and nearer 1.98 \(\text{Å}\) in alkyl derivatives). Bond angles are generally not so well known. Arsine has a structure similar to ammonia but the bonds are apparently more purely \(p\) bonds, since the angles between the As—H bonds are 91°35′, but in the pentavalent arsionic acids the angles are nearly tetrahedral in orientation. Bond energies have been calculated to be 32.1 kcal/mole for As—As, 58.6 kcal/mole for As—H, and 68.9 kcal/mole for As—Cl (Pauling, 1960). The van der Waals’ radius of arsenic is 2.0 \(\text{Å}\). The electronegativity value is 2.0 so that arsenic assumes a positive fractional charge in most of its bond dipoles.

<table>
<thead>
<tr>
<th>Bond length (Å)</th>
<th>Bond length (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td>As — H</td>
<td>1.52</td>
</tr>
<tr>
<td>As — C</td>
<td>1.94</td>
</tr>
<tr>
<td>As — O</td>
<td>1.87</td>
</tr>
<tr>
<td>As = O</td>
<td>1.69</td>
</tr>
<tr>
<td>As — Cl</td>
<td>2.18</td>
</tr>
<tr>
<td>As — Br</td>
<td>2.34</td>
</tr>
<tr>
<td>As — I</td>
<td>2.52</td>
</tr>
<tr>
<td>As = As</td>
<td>2.44</td>
</tr>
</tbody>
</table>
Determination

The usual qualitative and quantitative methods for the detection and analysis of inorganic arsenic (such as the Bettendorff, the Marsh, and the Gutzeit tests), frequently used in commercial and forensic work, are described in the standard texts, and it need only be mentioned that quantities as small as 0.1 µg of arsenic can be determined with considerable accuracy by modifications of the Marsh test wherein the deposited arsenic is titrated conductometrically. It is more pertinent for our present purposes to mention the excellent analytical procedures developed by Crawford in Edinburgh. Originally a technique was devised for the separation of arsenite and arsinite in biological material using sodium ethylxanthate and distribution in carbon tetrachloride (Crawford and Story, 1944). However, it was found that sodium ethylxanthate is not suitable for the aromatic arsinites and arsonates; for this purpose, ethane-1,2-diol in carbon tetrachloride proved to be satisfactory, the method being accurate for 2.µg arsenic (Crawford and Levy, 1946). Thus it is possible to separate and determine inorganic and organic trivalent and pentavalent arsenicals in biological material and this has been of use in studying the distribution and metabolism of such compounds (Crawford, 1947). A sensitive spectrophotometric test for arsenic has recently been described by Dal Cortivo et al. (1960) and is based on the evolution of arsine into a pyridine solution of silver diethylidithiocarbamate. Arsenic in tissues can be determined down to 1 µg% and in urine to 0.1 µg% by this method. It may be noted that a specific color reaction between oxophenarsine and 1,2-naphthoquinone-4-sulfonate is often useful in detecting this arsenical in tissues (Rosenthal, 1932 a). Arsenite and arsenate can be separated and determined by paper chromatography, and spot testing is available for the microdetermination of arsenic. Organic arsenicals may be determined by a spot test after conversion to arsenate by heating with lime and treatment with stannous chloride in HCl solution, with a sensitivity of around 1.4 µg. The most sensitive of all methods is based on activation analysis and quantities down to 0.001–0.01 µg can be determined in biological material (Christell and Sjöstrand, 1962).* The method involves neutron bombardment of the samples, separation of the As^{76}, and determination by β-counting or γ-spectrometry.

* This method allowed the determination of the arsenic content in the disinterred fragments of the sixteenth century King Erik XIV of Sweden for the purpose of providing evidence on the rumored poisoning by his brother.
The development of the concept that arsenicals affect biological systems through reaction with SH groups was briefly discussed in the introduction to this chapter. There are no other groups of biological importance with which the trivalent arsenicals readily react, and in this sense these inhibitors may be said to be quite specific.

Reactions with Thiols

The reaction of inorganic trivalent arsenic with sulfides has, of course, been known for a very long time. The first organic thioarsinite to be studied was arsenic trithiolacetic acid:

\[
\begin{align*}
S-\text{CH}_2-\text{COOH} \\
\text{HOOC-CH}_2-S-\text{As} \\
S-\text{CH}_2-\text{COOH}
\end{align*}
\]

in 1904, this being a crystalline substance forming a soluble sodium salt and easily prepared by reacting arsenic trioxide with thiolacetic acid in aqueous solution. Thioarsinites of the organic arsenicals were soon made and tested for activity against microorganisms. Labes (1929 a) obtained the thioarsinite formed by arsenious acid and cysteine (i.e., tricysteinylarsine) in crystalline form, and Johnson and Voegtlin (1930) obtained the same product by reacting arsenious chloride with cysteine at room temperature. By 1930 there was thus not only good evidence that both inorganic and organic trivalent arsenicals can react with various thiols, but some well-characterized thioarsinites had been prepared in pure form.

It is important to realize that the \( q \)-arsonates can also eventually react with thiols. Friedberger (1908) found that \( p \)-amino-\( q \)-arsonate when mixed with thiolacetic becomes highly toxic to mice and is an effective trypanocide. It was soon shown by Roehl (1909) that this is due to the reduction of the arsonate to the corresponding arsenoxide, which is, of course, active. However, it was noted that if thiolactate is in excess, some of the arsenoxide formed reacts to form a dithioarsinite:

\[
\begin{align*}
R-\text{AsO(OH)}_2 + 2 \text{HS-R'} &\rightleftharpoons R-\text{As}=\text{O} + \text{R'}-\text{S-S-R'} + 2 \text{H}_2\text{O} \\
\text{S-R'}
\end{align*}
\]

\[
\begin{align*}
R-\text{As}=\text{O} + 2 \text{HS-R'} &\rightleftharpoons R-\text{As} + \text{H}_2\text{O} \\
\text{S-R'}
\end{align*}
\]

The reduction proceeds in acidic but not appreciably in alkaline solutions. If cystine is mixed with an arsenoxide, cysteine is formed and the arsenical.
is oxidized if the medium is somewhat alkaline (Cohen et al., 1931). These facts emphasize the important role of the pH in determining the direction in which such reactions proceed. Barber (1929) claimed that thiols can reduce pentavalent arsicals in acid, neutral, or alkaline solutions, and that the resultant trivalent forms combine with excess thiol, as above. He found that the aryl thioarsinates formed with thiolacetate are in general crystalline, are quite soluble, and are not split by acid or alkali; however, they are oxidized in air in alkaline solutions and this is more rapid than for the parent compounds. Cohen et al. (1931) disagree with the mechanism postulated inasmuch as they had found no such reduction in alkaline media. Instead, they assume that around neutrality a β-arsonate combines with four molecules of a thiol to form an intermediate tetrathioarsonate, which is unstable and splits into two thioalkyl groups (which unite to form a disulfide) and the dithioarsinite. Such a reaction would seem kinetically unlikely and it might be suggested that even in neutral or alkaline solutions the reduction can proceed, since the reduced forms immediately react with SH groups, even though the equilibria under these conditions favor the oxidized form of the arsénale. The dithioarsinates are formed readily in most instances from the monosubstituted arsénals, and the monothioarsinates are difficult to obtain; e. g., simply mixing solutions of cysteine and arsenite results in a rapid precipitation of the dithioarsinite (Labes, 1929 b), and the product of the reaction of cysteine with oxophenarsine is the dicysteinyloxoophenarsine (Johnson and Voegtlín, 1930).

Surprisingly little appears to be known about the intimate mechanisms in the reactions of arsénals with thiols. How should one visualize the fundamental formation of a mercaptide from an arsénoxide, R—As=O? Is it a progressive addition and substitution, which may be written as:

\[
\begin{align*}
\text{OH} & \quad \text{S—R'} \\
R—\text{As=O} & \rightarrow R—\text{As} \\
& \quad \text{S—R'} \\
& \quad \text{S—R'}
\end{align*}
\]

or is it a simultaneous replacement of the O atom (in other words, does the monothioarsinite actually occur)? Or is it the arsonous acid form which reacts:

\[
\begin{align*}
\text{OH} & \quad \text{S—R'} \\
R—\text{As} & \rightarrow R—\text{As} \\
& \quad \text{OH} \\
& \quad \text{S—R'}
\end{align*}
\]

and, if so, what is the mechanism of the substitution? Eagle (1945) found that over a limited physiological pH range, there is little if any effect of pH on these reactions. It is well known that compounds such as R—AsCl₂
and $R_2-\text{AsCl}$ react rapidly with thiols, and this might point to the $R-\text{As(OH)}_2$ form as the reactive one. It is unlikely that an intermediate of the type $R-\text{As(OH)}_2-S-R'$ is involved, since Parker and Kharasch (1959) have shown that such unstable compounds are hydrolyzed to yield the corresponding arsonic acid. One would also like to know more about the relative rates at which various arsenicals react with different thiols. Aldridge and Cremer (1955) reacted three arsenicals with glutathione and dimercaprol, and determined the extent of the reaction by measuring the increase in the time for reduction of 2,6-dichlorophenolindophenol by the thiol (Table 6-2). It is seen that all the arsenicals react with dimercaprol faster than with glutathione; indeed, arsenite does not react with glutathione at all, a fact confirmed by Drummond and Stern (1960), who observed no mercaptide formation even at 100 mM arsenite. It is also interesting that di-\(\varphi\)-AsCl is the most reactive of the three, at least with the monothiol. Stocken and Thompson (1946 b) reacted lewisite with a variety of thiols and then determined how rapidly the resulting thioarsinites hydrolyze by measuring the rate of porphyrindin reduction (see accompanying tabulation), and these results well demonstrate the markedly greater sta-

<table>
<thead>
<tr>
<th>Monothiols</th>
<th>Decoloration time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysteine</td>
<td>0.25</td>
</tr>
<tr>
<td>Thiolactate</td>
<td>0.5</td>
</tr>
<tr>
<td>Aminothiophenol</td>
<td>0.83</td>
</tr>
<tr>
<td>Glutathione</td>
<td>2.5</td>
</tr>
<tr>
<td>Mercaptoethanol</td>
<td>3.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dithiols</th>
<th>Decoloration time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimercaprol</td>
<td>&gt;180</td>
</tr>
<tr>
<td>Dimercaptoethane</td>
<td>&gt;180</td>
</tr>
<tr>
<td>1,3-Dimercaptopropanol</td>
<td>&gt;180</td>
</tr>
<tr>
<td>1,3-Dimercaptopropane</td>
<td>&gt;180</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Decoloration time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle protein</td>
<td>3.0</td>
</tr>
<tr>
<td>Lens protein</td>
<td>3.5</td>
</tr>
<tr>
<td>Kerateine</td>
<td>6.0</td>
</tr>
</tbody>
</table>

bility of the compounds formed with the dithiols. The fairly rapid splitting of the monothioarsinites and the protein complexes indicate that most enzyme inhibitions by the arsenicals should be readily reversible if secondary changes in the protein have not occurred.
Table 6-2
Comparison of the Rates at Which Arsenicals React with Glutathione (GSH) and Dimercaprol (BAL)\(^a\)

<table>
<thead>
<tr>
<th>Thiol</th>
<th>Concentration (mM)</th>
<th>Compound</th>
<th>Concentration (mM)</th>
<th>Concentration ratio</th>
<th>Decoloration time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>GSH</td>
<td>0.05</td>
<td>Arsenite</td>
<td>1.8</td>
<td>36</td>
<td>118</td>
</tr>
<tr>
<td></td>
<td>0.051</td>
<td>$q$-AsO</td>
<td>0.89</td>
<td>17.4</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>0.051</td>
<td>Di-$q$-AsCl</td>
<td>0.2</td>
<td>3.9</td>
<td>125</td>
</tr>
<tr>
<td></td>
<td>0.06</td>
<td>$p$-MB</td>
<td>0.081</td>
<td>1.3</td>
<td>118</td>
</tr>
<tr>
<td>BAL</td>
<td>0.023</td>
<td>Arsenite</td>
<td>1.8</td>
<td>78</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>0.022</td>
<td>$q$-AsO</td>
<td>0.044</td>
<td>2</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>0.022</td>
<td>Di-$q$-AsCl</td>
<td>0.1</td>
<td>4.6</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>0.023</td>
<td>$p$-MB</td>
<td>0.081</td>
<td>3.2</td>
<td>38</td>
</tr>
</tbody>
</table>

\(^a\) The incubation of the arsenical and thiol prior to the addition of the indophenol dye was 5 min at pH 7.4 and 18-20°. (From Aldridge and Cremer, 1955.)
Peters (1955) has emphasized that the trivalent arsenicals should not all be considered as acting in the same manner. There are, for example, both monosubstituted (arsenoso-) and disubstituted (arsinoso-) compounds, and these have been shown to have different reactivities with different types of thiol. As an outcome of the work on the arsenical war gases, Peters had come to the conclusion that the monosubstituted arsenicals (such as \( q \)-AsO or oxophenarsine) form particularly stable compounds with dithiols where the SH groups are spatially arranged so that a ring can be formed, the most stable ring being a five-membered one. Such cyclic thioarsinites:

\[
\begin{align*}
R-\text{As}=\text{O} & + \quad \text{HS}-\text{C} & \rightarrow & \quad R-\text{As} \quad \text{S}-\text{C} & + \quad \text{H}_2\text{O} \\
\text{HS}-\text{C} & & & \text{HS}-\text{C} & \\
\end{align*}
\]

are more stable than those formed from two molecules of a monothiol:

\[
\begin{align*}
\text{HS} & - \text{R}' & \rightarrow & \quad \text{S} & - \text{R}' \\
R-\text{As}=\text{O} & + & \quad \text{HS} - \text{R}' & \rightarrow & \quad R-\text{As} & \quad \text{S} & - \text{R}' & + \quad \text{H}_2\text{O} \\
\text{HS} - \text{R}' & & & \text{HS} - \text{R}' & \\
\end{align*}
\]

as may be seen in the tabulation above. The problem of the disubstituted arsenicals had been latent in Peters' mind for many years, inasmuch as he had previously shown that differences in action exist between these and the monosubstituted arsenicals. Furthermore, it is impossible for the disubstituted arsenicals to form ring structures; instead they should react with monothiols:

\[
\begin{align*}
\text{R} & \quad \text{As} & - \text{OH} & + \quad \text{HS} - \text{R}' & \rightarrow & \quad \text{As} & - \text{S} & - \text{R}' & + \quad \text{H}_2\text{O} \\
\text{R} & & & \text{R} & \\
\end{align*}
\]

steric factors should be kept in mind since these may limit the reaction. For example, monosubstituted arsenicals may react with two molecules of a small monothiol, but in the case of bulky thiols, such as proteins, this may be impossible. Disubstituted arsenicals may be able to react with both SH groups of a dithiol but only if the arsenical molecules do not sterically interfere with each other. Peters and his collaborators have indeed found enzymes (e.g., isocitrate dehydrogenase and aconitase) which are inhibited by the disubstituted arsenicals but not by the monosubstituted arsenicals, while other enzymes (e.g., the \( \alpha \)-keto acid oxidases) are inhibited more readily by the monosubstituted arsenicals. The problem of
why the ring structure is more stable than the linear one for the thioarsinites has not been completely answered. In fact, whether such increased stability occurs has been questioned, since there is no resonance which can account for it (Eagle and Doak, 1951). Metal chelates possess special stability in part, because of entropy factors and a similar explanation may be involved here. Also, the steric factor must play a role in the case of most monothiols attaching to a single arsenic atom in pairs; in addition, electrostatic repulsion could contribute if ionized groups occur on the thiol.

Reactions with Proteins

There has been very little quantitative work on the binding of arsensicals to different types of protein. Indirect evidence indicates that arsensicals often react with proteins, but the mechanism of the binding is unknown. For example, the fact that serum albumin decreases the ability of oxophenarsine to inactivate trypanosomes shows that a complex is formed (Toyoshima, 1952). When radioactive arsenite is injected into rats, the relatively large amounts in the erythrocytes appear to be bound to the globin portion of hemoglobin, with little attached to the stroma (Hunter and Kip, 1941; Hunter et al., 1942). However, in other species much less occurs in the blood and little seems to be bound to either hemoglobin or serum proteins. If the liver proteins are fractionated on the basis of solubility, the arsenite is found not to be uniformly distributed between the various fractions (Lowry, et al., 1942). When rabbit blood is incubated in vitro with oxophenarsine, more arsenical is bound to hemoglobin than to the serum proteins or stroma; however, relatively more is bound to urease and the binding is about 3-fold tighter, suggesting the importance of SH groups (Fink and Wright, 1948 b). The protein SH groups are lowered about 80% in renal cortex, medulla, and the distal tubules following incubation of kidney sections with rather high concentrations of arsenite, the effect being as marked as with HgCl₂ or iodoacetate (Cafruny et al., 1955 a). These types of result, important as they are in various ways, do not provide much information on how the arsensicals react with proteins.

What evidence is there that arsensicals can react with protein SH groups? Rosenthal (1932 b) incubated various proteins with 1 mM oxophenarsine, and determined how much of the arsensical appeared in a dialysate (see accompanying tabulation). None of the purer proteins in their native state bound appreciable arsensical, but did so upon denaturation, which was interpreted as due to the release of SH groups. It was also shown that the nitroprusside reaction for SH groups declines and disappears when ovalbumin is incubated with oxophenarsine. This is not due to oxidation because no oxygen is taken up and cyanide does not restore the SH groups. Rosenthal calculated that 1 g crystalline ovalbumin binds 12.72 mg oxophenarsine; this is about 2.4 moles of arsensical per mole of protein,
assuming a molecular weight of 44,000 for ovalbumin. If 1 molecule of arsenical combines with 2 SH groups, this would indicate 4.8 reactive SH groups on ovalbumin. Since there are 5 cysteine residue, this checks quite satisfactorily. It is not known if the arsenical reacts with 2 SH groups on a single ovalbumin molecule or with SH groups on different molecules (the figures above indicate at least some intermolecular linking). In the experiments of Rosenthal, it is possible that some arsenical reacted with the native proteins but split off during the dialysis.

Stocken and Thompson (1946 a) studied the reactions of arsenicals with keratine (reduced keratin) and it was this work which originally led the Oxford group to postulate the cyclic thioarsinite structure. Keratine was titrated with lewisite at pH 7 until the free SH groups had disappeared; arsenite reacted less readily and a high concentration was required (even then all the SH groups were not reacted). The arsenical-proteins are soluble below pH 4 and above pH 5.4, exhibiting a minimum solubility around pH 4.6. When these derived proteins were dialyzed, it was found that the lewisite complex, is more stable: 20-hr dialysis caused a loss of 40% of the bound lewisite and 85% of the bound arsenite, while 40-hr dialysis gave a 53% loss of lewisite and a 100% loss of arsenite. Evidence that the binding is primarily through SH groups was obtained by showing that metakeratin (reoxidized keratine) binds only about one tenth as much arsenical as does keratine, and this is easily removed by dialysis, indicating nonspecific adsorption. Porphyrindin titrations carried out during the reaction indicated that the arsenicals combine with two SH groups. It is not known if a ring structure is formed or whether the binding is between different keratine molecules; the decreased solubility might possibly
indicate the latter. There is some evidence the arsenite can react with keratin under appropriate conditions by first reducing the disulfide groups to SH groups, as might occur in the binding of arsenite to hair, a phenomenon known for many years.

The reactivity of a particular SH group on a protein undoubtedly varies with the location of the SH group. The larger arsenicals might be sterically prevented from reaching certain SH groups, and electric fields around SH groups would influence the approach of charged arsenicals. The question whether two appropriately spaced SH groups must be present to ensure a tight binding of monosubstituted arsenicals, enabling a ring to be formed, cannot be definitely answered, although this is assumed in much work on enzyme inhibition. It seems to be true that arsenicals are generally more selective SH reagents, compared for example to the mercurials, and they are not useful substances for the titration of all reactive SH groups. This point will be taken up again in the section on enzyme inhibition, but it is likely that monosubstituted arsenicals do not usually react readily or strongly with single SH groups on proteins, although the evidence for this is not as convincing as one would like. Furthermore, it is doubtful if the organic arsenicals can react with SH groups from different protein molecules, since the bond angles, steric factors, and electrostatic repulsion would in most cases be unfavorable. Possibly two SH groups on the same protein, not normally adjacent, might react with a single arsical molecule if the protein structure is flexible, and this would perhaps alter the protein structure. Indeed, there is evidence that certain enzymes are not reactivated even though all of the arsical is removed, and this could be the result of irreversible structural alterations.

**INHIBITION OF ENZYMES**

The earliest workers to examine the effects of the arsenicals on enzymes unfortunately had only insensitive enzymes at their disposal. Thus emulsin, myrosin, pepsin, trypsin, diastase, and similar enzymes were found to be unaffected by arsenite (Boehm and Johannsohn, 1874), and this apparently supressed further work on enzymes for many years and led to statements that the actions of the arsenicals could not be explained on the basis of interference with enzymes. Several reports from 1904 to 1915 did not clarify the problem, since enzymes such as catalase (Senter, 1904; Santesson, 1915), “zymase” (Harden and Young, 1911), and succinate oxidase (Battelli and Stern, 1911) were found to be inhibited only by high concentrations of arsenite; low concentrations either had no effect or actually stimulated. More susceptible enzymes were found in the 1920’s — e. g., lipase (Rona and Bach, 1920), urease (Rona and György, 1920), maltase (Rona et al., 1922), and xanthine oxidase (Barry et al., 1928), but throughout this work
there was no definite concept of mechanism, although occasionally the idea was expressed that the inhibition might involve oxidation-reduction reactions. Meanwhile the importance of thiols and their reaction with arsenicals were being established, so when certain enzymes were found to have SH groups it was natural to examine inhibition in this light. The first implication of the role of SH groups in enzyme inhibition was apparently made by Bersin (1934) using papain. It was shown that inactive oxidized papain is activated by various arsenoxides, this presumably being due to a reduction of the S—S groups to SH groups (Bersin and Köster, 1935):

$$\text{S} + \text{R—As=O} + 2 \text{H}_2\text{O} \rightleftharpoons \text{S—SH} + \text{R—AsO(OH)}_2$$

However, when fully active reduced papain is treated with the arsenoxides an inhibition is observed (see accompanying tabulation). Actually, no direct evidence that the inhibition is due to mercaptide formation was provided, but Maschmann (1935 b) showed that the inhibition of liver cathepsin is associated with a reduction in the nitroprusside reaction, indicating that the enzyme SH groups react with the arsenical. Although it has generally been assumed since then that most enzyme inhibitions are the result of a reaction of the arsenical with a necessary enzyme SH group, in very few cases has this been conclusively demonstrated by titration of the SH groups.

**Possible Mechanisms of Inhibition**

Arsenical inhibition may or may not involve SH groups. If it does, the SH groups do not have to be on the apoenzyme, but can be on the substrate, a cofactor, or some intermediate. The inhibition of cysteine desulphydrase by arsenite (Lawrence and Smythe, 1943; Powning, 1954) might be due in part to the reaction with cysteine, but it was also postulated that mercaptopyruvate is an intermediate in the reaction:

<table>
<thead>
<tr>
<th>Form of papain</th>
<th>Arsenical</th>
<th>Activity</th>
<th>% of full activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxidized</td>
<td>—</td>
<td>0.09</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td>$p$-NH$_2$-$p$-AsO (1 mM)</td>
<td>0.37</td>
<td>39.4</td>
</tr>
<tr>
<td>Reduced</td>
<td>—</td>
<td>0.94</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>$p$-NH$_2$-$p$-AsO (1 mM)</td>
<td>0.66</td>
<td>70.2</td>
</tr>
</tbody>
</table>
so that part of the inhibition might be attributed to removal of this intermediate by the arsenite, which is supported by the fact that NH₃ production is inhibited much less than the formation of H₂S. In lipoate-dependent enzyme systems it is often difficult to determine if the site of arsenical inhibition is the apoenzyme or the lipoate (see page 651). The pentavalent arsenicals may oxidize SH groups and occasionally inhibition is reversed by reducing agents. Thus Itoh et al. (1939) found that serum lipase inhibited by atoxyl can be partially reactivated by cyanide.

Arsenite may affect enzyme activity by a nonspecific anionic action, as in the stimulation of salt-free fumarase (Massey, 1953 a), or by a more specific competitive inhibition with an anionic substrate, as in the depression of uitrite oxidation in *Nitrobacter* (Butt and Lees, 1960). Arsenite might act as a reductant in certain oxidizing systems and thus inhibit. The formation of δ-chlorolevulinate from β-ketoadipate and Cl⁻ by a soluble chlorinase from *Caldariomyces* possibly involves a chlorinium ion, arising by oxidation of Cl⁻ by oxygen, and the inhibition by arsenite was suggested as resulting from its reducing power rather than a reaction with SH groups (Shaw and Hager, 1959). The organic arsenicals can inhibit by a mechanism more related to their total structure, either competing with a substrate of similar properties or simply binding at or near the active site through a variety of forces. Benzoates and other aromatic substances often inhibit quite effectively and there is no reason that aromatic arsenicals cannot act in the same manner. Some likely instances of this type of inhibition will be mentioned in connection with specific enzymes (see page 647). If inhibition of an enzyme is observed at arsenical concentrations only around 1 mM or higher, at least one must seriously consider these other mechanisms, but even potent effects do not establish an SH mechanism.

**Use of Arsenicals to Detect Monothiol and Dithiol Groups on Enzymes**

The arsenoso and arsinoso compounds have come to be tools for the detection of single SH groups and vicinal pairs of SH groups on enzymes, inasmuch as arsenoso (or arsenoxide) derivatives are supposed to react with two close SH groups preferentially while the arsinoso (or arsinous acid) derivatives are supposed to react only with single SH groups. The basis for this is principally the work on the reactions of arsenicals with simple monothiols and dithiols, as discussed previously (page 612). Various criteria are used for concluding that an enzyme has single or paired SH groups. The initial distinction was made by Lotspeich and Peters (1951) in their study of isocitrate dehydrogenase, which responds to arsenicals quite differently than the α-keto acid dehydrogenases. The latter are more sensitive
to \( q - As = O \) than to \( q_2 = As - Cl \) — e.g., the \( \alpha \)-ketoglutarate oxidase of rat liver is some 11 times more sensitive, and the pyruvate oxidase some 40 times more sensitive (Aldridge and Cremer, 1955) whereas isocitrate dehydrogenase is more sensitive to \( q_2 = As - Cl \) than to \( q - As = O \). It was concluded that isocitrate dehydrogenase can form a stable monothioarsinite but not a cyclic thioarsinite, and hence possesses a single SH group at or near the active site. Aconitase was found to behave similarly (Peters, 1955), since it is readily inhibited by \( q_2 = As - Cl \) but is unaffected by lewisite. The creatine kinase of rabbit muscle is unaltered by 0.25 mM lewisite but is inhibited 82\% by the same concentration of \( q_2 = As - Cl \) (Rosenberg and Ennor, 1955) and the arginine kinase from crayfish muscle is insensitive to arsenite but is strongly inhibited by \( q_2 = As - Cl \) (Morrison et al., 1957), so that on this basis these enzymes fall into the same monothiol class.

A number of enzymes have recently been classified as monothiol or dithiol\(^\dagger\) from their responses to arsenicals. In some cases it has been concluded that an enzyme is in the dithiol class simply because it is strongly inhibited by arsenite or the arsenoxides: this is the case with dihydrolipoate dehydrogenase (Massey and Veeger, 1960), succinic semialdehyde dehydrogenase (Albers and Koval, 1961), glycine reductase (Stadtman, 1962), and UDP-translucuronylase (Storey, 1964). Sometimes evidence is adduced from the ability or inability of simple monothiols and dithiols to protect or reverse the inhibition. Thus Storey (1964) felt that the inability of glutathione or cysteine to completely reactivate arsenical-inhibited UDP-translucuronylase supports the dithiol nature of this enzyme, while Lakshmanan et al., (1964) doubted if dithiol groups occur on monkey liver aldehyde oxidase because the inhibition by arsenite can be prevented only by high concentrations of dimercaprol. Some have deduced a monothiol group because the enzyme is inhibited by a mercurial but not by arsenite or an arsensoxide; this is the situation with aspartase, which Ellfolk (1953) designated as a monothiol since it is much more strongly inhibited by \( p \)-mercuribenzoate than by \( q - As = O \). Cadmium ion is also used as a dithiol detector and Peters and Sanadi (1961) felt that, although xanthine oxidase is well inhibited by arsenite, the failure of cadmium to inhibit is evidence against the dithiol nature of the enzyme. There is thus a good deal of confusion because of the lack of accepted criteria for distinguishing between monothiol and dithiol enzymes.

Since this is a very important aspect of the use of arsenicals as enzyme inhibitors, it will be well to consider the problem in greater detail. Let us

\* The nucleus of the phenylarsenoxides will be designated as \( q - As = O \) and of the diphenylchloroarsines as \( q_2 = As - Cl \) for convenience in the following discussion.

\dagger An enzyme will be designated as a dithiol when it is thought to have two vicinal SH groups, i.e., two SH groups sufficiently close to form a cyclic thioarsinite complex.
write the various possible reactions which may occur between the different arsenicals and enzymes:

A. \( \text{R—As}=\text{O} + \text{HS—E} \rightleftharpoons \text{R—As—S—E} + \text{OH} \)

B. \( \text{R—As}=\text{O} + 2 \text{HS—E} \rightleftharpoons \text{R—As—S—E} + \text{H}_2\text{O} \)

C. \( \text{R}_2\text{As—Cl} + \text{HS—E} \rightleftharpoons \text{R}_2\text{As—S—E} + \text{HCl} \)

D. \( \text{R—As}=\text{O} + \text{HS—E} \rightleftharpoons \text{R—As—S—E} + \text{H}_2\text{O} \)

E. \( \text{R}_2\text{As—Cl} + \text{HS—E} \rightleftharpoons \text{R}_2\text{As—S—E—SH} + \text{HCl} \)

F. \( 2 \text{R}_2\text{As—Cl} + \text{HS—E} \rightleftharpoons \text{R}_2\text{As—S—E—S—As}=\text{R}_2 + 2 \text{HCl} \)

It is generally considered that reactions C and D represent the mechanisms by which potent inhibition is produced. It is not known whether reaction A can occur, but if it does it probably by itself does not lead to a very tight binding, at least relative to the cyclic thioarsinite formed in reaction D. Reaction B appears to be unlikely because of steric and electrostatic factors in holding two protein molecules when the bond angle from arsenic is fixed. Reaction F should become more and more unlikely as the \( R \) groups become larger or more charged, again because of steric and electrostatic factors, providing the \( \text{SH} \) groups are close together as is assumed here. Thus we are left with reactions C, D, and E as being the most important for potent inhibition of enzymes in general. The following points may now be made.

(1) Potent inhibition by \( \text{R—As}=\text{O} \) would not be adequate evidence for a dithiol since other factors might be involved. Oxophenarsine, for example, might inhibit well due to electrostatic forces or hydrogen bonds from the amino or hydroxy groups to the enzyme, as well as less specific forces between the benzene ring and the enzyme, which might stabilize a complex such as that formed in reaction A. Of course, the problem always arises as to exactly what should be considered a potent inhibition; e.g., some workers have argued for \( \text{SH} \) groups on the basis of moderate inhibition by arsenicals at 1–10 mM, whereas Dawson (1956 b) stated that phospholipase B probably contains no important \( \text{SH} \) groups despite a 44% inhibition by 0.5 mM \( \text{R}_2\text{As—Cl} \) (it is interesting that iodoacetate and \( p\)-MB do not inhibit well but \( \text{Hg}^{++} \) does).
(2) Failure to observe a potent inhibition by \( R-\text{As}=\text{O} \) would not prove the absence of a dithiol group because of various factors which might impede the binding. It seems that in some respects arsenite is not always the best test substance since usually the substituted arsenoxides inhibit much more potently. The reason for this is not known but may involve the small size of the arsenite, in that it will not interfere with the binding of the substrate unless the SH groups are actually in the active site, whereas larger R groups would be more likely to block sterically. It is also probable that the arsenite is not as reactive with enzyme SH groups, since it usually does not react as rapidly with simple thiols as do the substituted arsenoxides.

(3) Inhibition by \( R_2-\text{As}-\text{Cl} \) would not prove that there is only a single SH group on the enzyme, since there is no apparent reason why it would not react with one SH group of a pair just as well as with a single SH group, as in reaction E.

(4) Data from protection and reversal experiments often are completely valueless for determining the nature of the reaction with the enzyme. Just as protection or reversal by a thiol doesn’t prove that an inhibitor reacts with an enzyme SH group, protection or reversal by a dithiol doesn’t prove that an enzyme dithiol is involved. Such experiments only give some indication of the relative affinities of the enzyme and the reverser for the arsenical. Monothiols will generally be ineffective in preventing arsenical inhibitions since in many cases stable complexes are not formed, and dithiols, such as dimercaprol, will generally be effective whatever the nature of the reaction with the enzyme. There are undoubtedly cases in which an arsenical is bound to dimercaprol more strongly than to an enzyme, and this may be true even though the enzyme has a dithiol group, so that significant reactivation by dimercaprol certainly does not indicate whether an enzyme is of the dithiol type or not. It must also be remembered that some enzymes are inhibited by dimercaprol and this action may obscure any reactivation produced, and that the degree of reactivation will depend on whether the enzyme has undergone irreversible changes as a result of arsenical binding. It is believed that only under exceptional circumstances can protection or reversal data be used as evidence for the nature of enzyme SH groups.

(5) Occasionally the presence of a simple thiol markedly increases the inhibition by an arsenical (see page 645). Thiol transacylase is not inhibited at all by 5 mM arsenite, but in the presence of 2-mercaptoethanol the enzyme is depressed 73\% (Alberts et al., 1963). It was assumed, and probably rightly so, that these data do not indicate the enzyme to be of the dithiol type. Succinic semialdehyde dehydrogenase is inhibited 50\% by 4 mM arsenite, but with 2-mercaptoethanol it requires only 0.05 mM
arsenite to inhibit 50% (Albers and Koval, 1961). This was interpreted as indicating the presence of a dithiol group. It is useless to try to decide between the conclusions from such results until we know exactly why the thiol potentiates the inhibition. We shall discuss this matter in a later section, but it is interesting to speculate here that such behavior may point to a monothiol type of enzyme, since now reaction A above may be modified to:

$$\text{S—E}$$

$$\text{R—As=O + HS—E + HS—T} \rightleftharpoons \text{R—As} + \text{H}_2\text{O}$$

$$\text{S—T}$$

where T—SH is a simple thiol, which may to some extent stabilize the complex and make unnecessary another SH group on the enzyme. If this explanation is true, inhibition by $R_2=\text{As—Cl}$ should never be potentiated by thiols, but I know of no experiments on this point.

Summarizing the situation, it is likely that the original criterion applied by Lotspeich and Peters (1951), namely, the relative susceptibilities of enzymes to monosubstituted (R—As=O) and disubstituted (R_2=As—Cl) arsenicals, is the most valid to judge whether an enzyme possesses single or paired SH groups, but from what we have said it is clear that even this is not an infallible test. What shall we say about those enzymes which are inhibited approximately equally by these two types of arsenical? This is the case with urease (Rona and György, 1920), cholinesterases (Mounter and Whittaker, 1953), liver succinate oxidase (Aldridge and Cremer, 1955), and several other enzymes. Should we conclude that possibly there are two SH groups but farther apart than would provide maximal stability for a cyclic structure? Or that reaction E above can lead to as stable a complex as reaction D due to interactions with the rest of the molecule? These questions cannot be certainly answered until we have more information of the relative inhibitions produced not only by series of arsenicals but also by other inhibitors reacting with SH groups. Unfortunately not a single enzyme has been subjected to an extensive group of arsenicals under the same experimental conditions, as may be seen from perusal of Table 6-3, and the answers we are looking for cannot be found from simple comparisons of the effects of arsenite or an arsinoxide with those of $\varphi_2=\text{As—Cl}$.

**Stimulation of Enzymes by Arsenicals**

Arsenicals in common with other SH reagents occasionally increase enzyme activity. In addition to the instances cited in Table 6-3, catalase is stimulated 45% by 5.5 mM cacodylate and initially by 7.4—11 mM arsenite (Santesson, 1915), dihydrofolate reductase is stimulated 17% by 10 mM arsenite (Blakley and McDougall, 1961), and the oxidation of $\alpha$-
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| Human erythrocytes | Oxophenarsine | 10 | 32 |

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**INHIBITION OF ENZYMES**
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<td>Concentration (mM)</td>
<td>% Inhibition</td>
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ketoglutarate by rat liver mitochondria is stimulated around 10\% by 0.033 mM arsenite (Corwin and Schwarz, 1963). Possible mechanisms for such stimulation were discussed in connection with the mercurials (page II-815) but, aside from the reduction of inactive oxidized papain by arsenoxydes (Bersin, 1934), the nature of the stimulations observed is unknown. J. S. Roth (1958) postulated that the activation of ribonuclease by phenylarsenoxide may be related to the removal of a naturally occurring inhibitor that is bound to the enzyme during extraction, but it is also possible that active ribonuclease is released from lysosomes. It is interesting that the oxidation of glutathione by mouse kidney homogenate is stimulated by 2 mM arsenite (Ames et al., 1946). Since it is generally assumed that arsenic does not react with glutathione, one must conclude that the action is on the enzyme or on a natural inhibitor.

The activation of myosin ATPase by butarsen may be especially important because it relates this enzyme to mitochondrial ATPase and oxidative phosphorylation (Blum and Sanadi, 1964). When butarsen is added to myosin ATPase along with ATP there is no effect at 0.33 mM, but in the absence of ATP there is a rapid activation, the rate approximately doubling in 200 sec. This effect is potentiated by 2-mercaptoethanol, which has no action of its own, while cysteine and thioglycolate prevent the activating action of butarsen. The ITPase activity, on the other hand, is inhibited by butarsen. Dimercaprol abolishes the action of butarsen on both ATPase and ITPase. Blum and Sanadi suggest that the 2-mercaptoethanol may react with the butarsen and that this complex penetrates to the site of ATPase activity to combine with an SH group there (see page 659), but the reason for the activation is not clear. Possibly structural changes near the active center of myosin are induced and facilitate access of ATP to its hydrolytic site, as has been postulated for the stimulation by 2,4-dinitrophenol.

**Potentiation of Arsenical Inhibition by Thiols**

Thiols usually reduce the actions of SH reagents on enzymes, but quite often the opposite happens in the case of the arsenicals, as we have already seen for succinic semialdehyde dehydrogenase and thiol transacylase (page 620), and in the activation of myosin ATPase in the preceding paragraph. Some further examples will illustrate the nature of this phenomenon. The aldehyde dehydrogenases from yeast and liver are unaffected by arsenite alone, but inhibition occurs readily when 2-mercaptoethanol is present (Jakoby, 1957, 1958). The enzymes are somewhat stimulated by the thiol alone, but arsenite brings the activity far below the control level without the thiol. The pyruvate oxidase from Acetobacter melanogenum is depressed very little by 1 mM arsenite, but in the presence of glutathione the inhibition is almost complete (Bone and Hochster, 1960). If $\beta$-hydroxy-
butyrate apodehydrogenase is incubated with lecithin and a thiol it becomes active; the complex with cysteine is unaffected by 1 mM arsenite while the dimercaprol complex is inhibited completely (Jurtshuk et al., 1963). This phospholipid complex is probably the form present in mitochondria and it is interesting to speculate whether the lecithin interferes with the access of the arsenite to the active site unless dimercaprol is present. We shall see that certain electron transport systems behave in the same way (page 659) and lipid there may also play a role in restricting certain polar inhibitors from getting to the site at which they would normally react when the enzymes are purified.

The mechanisms involved in the thiol potentiation of arsenical inhibitions are unknown. Most workers have assumed that the arsenical-thiol complex can better penetrate to the functional SH groups perhaps because it is more lipid-soluble. Another possibility is the formation of a ternary complex of arsenical-thiol-enzyme, as discussed previously. A further explanation, which seems to have been generally ignored, is the simple reduction of S—S to SH groups in a region near the active site; the SH groups need not be involved in the catalysis but, when combined with arsenical, would interfere with the approach of the substrate to the active site. On the other hand, one must bear in mind the fact that only certain thiols are potentiating for a particular enzyme. It is quite certain that some enzymes alternate between the S—S and SH forms during activity — i.e., these groups participate in the electron transfer — as in various metalloflavoenzymes concerned with NAD(P)H oxidation or in systems such as sulfite oxidase (Fridovich and Handler, 1956). First, oxidation of SH groups to the S—S form implies that the SH groups are quite close and hence might react readily with the monosubstituted arsenicals. Second, it would cause the susceptibility of the enzyme to vary with the state of the SH groups. This is perhaps why it has been found that addition of substrate occasionally potentiates the inhibition by an arsenical. Arsenite has no effect on dihydrolipoate dehydrogenase but in the presence of NADH the inhibition is marked (Searls and Sanadi, 1960 a). This was explained as a reduction of enzyme S—S groups to SH groups by the NADH through FAD. Similar results were obtained with $\alpha$-ketoglutarate dehydrogenase with NAD as the acceptor, although no potentiation of arsenite inhibition occurs when an indophenol dye is used as the acceptor (Searls and Sanadi, 1960 b). It was further shown that the enzyme exhibits paired SH groups after incubation with NADH (Searls et al., 1961). Glutathione reductase behaves in the same fashion in that the activity is unaffected by 1–10 mM arsenite in the absence of NADPH, whereas strong inhibition is seen when NADPH is added with the arsenite (Mapson and Isherwood, 1963). The enzyme was pictured as existing in three states in equilibrium — E—SH, E=S, and E—S—S—E — and NADPH was considered as generating the
E—SH form, which is susceptible to inhibition by arsenite, N-ethylmaleimide, and p-mercuribenzoate. These results have bearing on the effects of arsenicals in vivo, since the susceptibilities of the metabolic systems may well depend on the redox states of certain enzymes and hence on the supply of substrates and oxygen. Other instances of potentiation of arsenical inhibition are not so clearly understood. Arsenite depresses succinate dehydrogenase activity more strongly when 0.01 mM Ca\textsuperscript{++} is added (Kushnarev and Blagoveshchenskii, 1961), and the oxidation of glutamate by Vigna seedling mitochondria is insensitive to arsenite unless AMP is added (Das and Roy, 1962). Both of these effects may be related to alterations in the equilibrium between S—S and SH groups. For example, AMP stimulates glutamate oxidation and could thus shift this equilibrium.

**Types of Inhibition Observed with the Arsenicals**

Arsenical inhibitions may be recorded as either competitive or noncompetitive, depending on how the tests are made and how tightly the arsenical is bound to the enzyme. Most inhibitions are presumably due to the arsenical reacting with SH groups at or near the active center, and it is very commonly observed that the enzyme is protected by its substrate. If, however, the arsenical is bound tightly to the enzyme, the final equilibrium inhibition will not depend to any extend on the substrate. Thus one can obtain competitive, noncompetitive, and mixed kinetics from the same system if the determination of the inhibition is done at different times. Thus Peters and Sanadi (1961) showed that xanthine oxidase is protected against arsenite by the substrate, but that the final inhibition is formally noncompetitive with respect to xanthine. Competitive inhibition has been reported. The inhibition of d-amino acid oxidase by butarsen has been claimed to be competitive with respect to alanine (Frisell and Hellerman, 1957) but, since apparently only 15 min was allowed for the reaction, it is questionable if equilibrium conditions had been reached, so that only a competitive substrate protection was measured. On the other hand, the point was made that butarsen may be inhibiting by virtue of its benzoate-like structure, rather than as an SH reagent, especially when FAD is present to protect certain SH groups. Flamm and Crandall (1963) report that the inhibition of homogentisate oxidase by lewisite is noncompetitive with respect to substrate but competitive with respect to Fe\textsuperscript{++}, and the double-reciprocal plots obtained certainly seem to be beyond objection. Since the Fe\textsuperscript{++} and the arsenical may react with the same SH group, true competitive inhibition is not so surprising. Liver aldehyde oxidase is inhibited competitively by arsenite and, since the \( K_i \) of 3 mM indicates a rather low affinity of the enzyme for the arsenical, a true competitive situation could exist here (Palmer, 1962). On the other hand, rabbit liver aldehyde oxidase is inhibited competitively by arsenite with \( K_i = 0.0063 \)
of a certain nitrite cholinesterase, that been in inhibition, may be complicated by the shift in the S–S and SH group ratio, as we have seen for certain oxidizing enzymes. When the presence of the substrate favors the inhibition, one cannot immediately conclude that uncompetitive (coupling) inhibition occurs, i.e., the arsenical combines with the ES complex. Thus the inhibition of S-acetoacetyl-d-thiolethiooctanoate thioesterase by arsenite requires the presence of the substrate; since arsenite reacts with neither the enzyme nor the substrate alone, it was suggested that the reaction is with the ES complex (Drummond and Stern, 1961). This may well be so, but it may also imply that SH groups become available during the catalysis. Mutual depletion kinetics must also be expected in many arsenical inhibitions, and evidence for this is to be found in the results of Rona et al. (1922) on the inhibition of maltase by methylarsenoxide, and of Stoppani and Brignone (1957) on succinate dehydrogenase inhibited by methylarsenoxide and oxophenarsine.

Protection of an enzyme by a substrate, coenzyme, or cofactor implies that the inhibitor reacts with the substrate site or with some group within the radius of steric or electrostatic influence of the bound substrate, providing the protector does not directly react with the inhibitor, or merely stabilize the enzyme structure against secondary inactivation induced by the inhibitor. Some further examples of substrate protection will be mentioned: the inhibition of muscle succinate dehydrogenase by arsenite (Bergsterrman and Mangler, 1948), the inhibitions of urease, succinate dehydrogenase, and choline dehydrogenase by oxophenarsine (Gordon and Quastel, 1948), the inhibition of pyruvate decarboxylase by oxophenarsine (Stoppani et al., 1952), and the inhibition of yeast fumarase by Melarsen (Favelukes and Stoppani, 1958). The mechanisms of these protections are not known but it is generally assumed that they point to a binding of the arsenical at the substrate site. Protection by a coenzyme similarly suggests a common binding region, as the protection of aldehyde dehydrogenases by NAD and NADP observed by Stoppani and Milstein (1957 a, b). Other types of protection present greater difficulties in interpretation; for example, the protection of the malate oxidative decarboxylase by Mn^{2+} when arsenite is the inhibitor (Rutter and Lardy, 1958). Finally, we have protection against arsenical inhibition by more readily reversible inhibitors, such as
the protection of succinate dehydrogenase by malonate (Barron and Singer, 1945) and the protection of pyruvate decarboxylase against arsenoxides by \textit{p-mercuribenzoate} (Stoppani \textit{et al.}, 1953).

\textbf{Kinetics of Arsenical Inhibition}

The rates at which arsenicals inhibit enzymes may be fast or slow. Gordon and Quastel (1948) noted that the inhibition of urease by various arsenoxides does not increase with time and concluded that equilibrium is achieved very rapidly, and similar results have been obtained by Montgomery \textit{et al.} (1956) on rat heart $\alpha$-ketoglutarate oxidase inhibited by arsenite, and by Drummond and Stern (1961) on their liver thioesterase inhibited by arsenite. On the other hand, succinate oxidase is inhibited by $p$-amino-phenylarsenoxide much more slowly, 30–60 min being required for the maximal effect (Fig. 1-12-12) (Slater, 1949). $\beta$-Amylase is also inhibited slowly by the same inhibitor, the inhibition still increasing at 30 min (Ghosh, 1958). The dihydrolipoate dehydrogenase from spinach is little inhibited by 1 mM arsenite at 5 min but is almost completely inhibited by 15 min (Basu and Burma, 1960). Unfortunately the kinetics of arsenical inhibition have never been studied in sufficient detail to be interpreted, nor have the rates of development of the inhibitions been correlated with \textit{SH} titrations or reversibility experiments. However, it is important to realize that enzymes differ quite markedly with respect to their inhibition kinetics, and it is evident that even simple rate studies would often enable arsenical inhibitions to be understood better and applied with greater confidence to \textit{in vivo} effects.

\textbf{Effects of pH on Arsenical Inhibition}

Much of the work on the variation of enzyme inhibition with pH has been done with enzymes for which an \textit{SH} group mechanism is not certain. The high sensitivity of certain lipases to atoxyl is not explained, but Rona and Haas (1923) reported that minimal inhibition is seen around pH 6.9, more at pH 6.57, and most at pH 7.63, using human kidney lipase, whereas Rothschild (1929) found the inhibition of liver lipase by arsenite to be generally increased moderately as the pH is raised from 6.59 to 7.59. The activation of fumarase by arsenite is probably unrelated to \textit{SH} groups and here the activation is absent at pH 6.5 and increases rapidly up to pH 7.8 (Fig. 6-1) (Massey, 1953 a). The inhibitions of cholinesterases also increase with the pH (Fig. 6-2) (Mounter and Whittaker, 1953). It is not easy to interpret these results. Are the changes related to ionization of the arsenious acid, or of enzyme groups? The anion-type activation of fumarase might be explained on the basis of the arsenious acid ionization, but how does this fit in with the reported $\textit{pK}_a$ of 9.22, and why does the activation
begin at the same pH with all the substances tested, irrespective of their \( pK_a \)’s? It certainly seems unlikely that the marked rise in the inhibition of cholinesterase around pH 5.4 is related to ionization of arsenous acid. Is the reaction of arsenicals with SH groups accelerated by a rise in the pH?

Eagle (1945) reported that pH has little effect on the reactions between

![Graph](image)

**Fig. 6-1.** Activation of fumarase by various anions. (A) Absence of added anions; (B) selenate (30 mM) or sulfate (25 mM); (C) arsenite (10 mM); (D) citrate (10 mM); (E) borate (200 mM); (F) phosphate (60 mM); (G) arsenate (50 mM); (H) chloride (100 mM).

(From Massey, 1953. a)

organic arsenicals and simple thiols, and we know nothing about the effect of pH on the reactivity of protein or enzyme SH groups. The inhibition of \( \beta \)-amylase seems to involve SH groups because of the potent inhibition by the mercurials, and here there is also an elevation of the effect of \( p \)-aminophenylarsenoxide as the pH is changed from 5.5 to 7.2 (Ghosh, 1958), so that this might indicate a greater SH group reactivity at the higher pH, unless it is a matter of the ionization of the \( p \)-amino group; arsenite does not inhibit this enzyme. Both Barron and Singer (1945) and Mounter and Whittaker (1953) noted that phenylarsenoxide-enzyme complexes tend to dissociate when the pH is raised, this leading to reactivation of the enzymes. Although this corresponds to the observation that the As—S bond is more readily hydrolyzed under alkaline conditions it is, difficult to understand in view of the fact that inhibitions generally rise with the pH. There are some intriguing problems here which might repay further investigation.
Inhibition of α-Keto Acid Oxidases

The discovery by Krebs in 1933 that arsenite causes accumulation of α-keto acids in kidney slices led to the postulate that the site of block is on the oxidases for these acids, and such inhibition was confirmed by workers using different tissue preparations (see Table 6-3). Peters et al. (1946) particularly emphasized the sensitivity of these oxidases and felt that the arsénicals can, under the proper circumstances, exert quite specific effects on pyruvate and α-ketoglutarate oxidation. Most of this early work was done with various breis and dispersions, and it was not until the α-keto acid oxidases were more highly purified and their components elucidated around 1951-1952 that the mechanism of the inhibition could be approached. At first it was thought that the enzyme SH groups are the site of attack; when coenzyme A was found to be involved, it was suggested as the site of inhibition (Sanadi et al., 1952); when the role of lipoate was established, it was evident that the adjacent SH groups would allow the formation of a stable cyclic thioarsinite (Gunsalus, 1953). It has been generally assumed since that time that arsénicals inactivate the lipoate, and hence prevent the transfer of the acyl groups to coenzyme A as well as the uptake of oxygen for reoxidation of reduced lipoate.

The detailed mechanism of the complex α-keto acid oxidases has not yet been elucidated and the schemes presented do not completely explain
the results obtained with arsenicals. Many of the intermediates in the sequence are hypothetical. One scheme involves the following reactions:

\[
\begin{align*}
\text{Pyruvate} + \text{TPP} & \rightarrow \alpha\text{-hydroxyethyl-TPP} + \text{CO}_2 \\
\alpha\text{-Hydroxyethyl-TPP} + \text{lipS}_2 & \rightarrow \text{acetyl-TPP} + \text{lip(SH)}_2 \\
\text{Acetyl-TPP} + \text{CoASH} & \rightarrow \text{acetyl-S-CoA} + \text{TPP} \\
\text{Lip(SH)}_2 + \text{NAD}^+ & \rightarrow \text{lipS}_2 + \text{NADH} + \text{H}^+ \\
\text{Pyruvate} + \text{CoA-SH} + \text{NAD}^+ & \rightarrow \text{acetyl-S-CoA} + \text{NADH} + \text{H}^+ + \text{CO}_2
\end{align*}
\]

The acetyl-CoA enters the cycle or is metabolized in other ways, while the NADH is oxidized through the usual metallo-flavo-cytochrome system. Another scheme also involves the acetylation of lipoate:

\[
\begin{align*}
\text{"Aldehyde-TPP"} + \text{lipS}_2 & \rightarrow \text{acetyl-S-lipSH} + \text{TPP} \\
\text{Acetyl-S-lipSH} + \text{CoASH} & \rightarrow \text{lip(SH)}_2 + \text{acetyl-S-CoA}
\end{align*}
\]

where the dihydrolipoate is oxidized via NADH by a dihydrolipoate dehydrogenase. Various electron acceptors other than NAD can function in the system.

The inhibition of these systems will depend on the method by which the activity is measured. If lipoate is inactivated by an arsenical, one would expect inhibition of the \(O_2\) uptake and of the anaerobic dismutation of pyruvate, but not much inhibition of \(CO_2\) formation (decarboxylase activity), since lipoate does not function in this early step. These results have, in general, been obtained; the inhibition of pyruvate decarboxylation occurs only at higher concentrations of arsenical than are required to block \(O_2\) uptake (Table 6-3 and Fig. 6-3). The difficulties arise when artificial electron acceptors are used. Although the reduction of NAD is potently inhibited by arsenite (Sanadi et al., 1959; Goldman, 1959 a) in both the pyruvate and \(\alpha\)-ketoglutarate oxidase systems, there is usually much less inhibition on the reduction of other acceptors. Methylene blue reduction is inhibited moderately (Peters et al., 1946), but the reduction of ferricyanide is not inhibited up to 10 mM arsenite (Sanadi et al., 1952, 1959). When 2,6-dichlorophenolindophenol is used as the acceptor, only 4% inhibition from 30 mM arsenite was reported by Moyed and O'Kane (1956) with the *Proteus* pyruvate oxidase, while no inhibition by 0.5 mM arsenite was observed on the \(\alpha\)-ketoglutarate oxidase from rat heart, at which concentration \(O_2\) uptake is abolished (Montgomery et al., 1956). The failure to inhibit with this dye as acceptor is surprising because is was assumed from various evidence that lipoate is involved in its reduction. How can these results be reconciled with the reaction sequences such as discussed above? Both schemes postulate that the primary reduction involves lipoate. One must first admit that lipoate has not been directly demonstrated to be
INHIBITION OF ENZYMES

the site of arsenical inhibition, nor has the reaction between arsenicals and lipoate been adequately studied, and there is evidence that perhaps another site must be sought. There is reason to believe that arsenite inhibits dihydrolipoate dehydrogenase by reacting with SH groups on the apoenzyme and that these SH groups are instrumental in the oxidation of the reduced lipoate (Basu and Burma, 1960; Lusty, 1963; Matthews and Reed, 1963).

It is also interesting that very small quantities of lipoate prevent the inhibition of the pyruvate oxidase system by arsenite (Grunert and Rohdenburg, 1960). This does not seem to be due to a reaction with the arsenite since lipoate at only 1/500–1/100 the arsenite concentration is effective. With regard to the failure of arsenicals to inhibit the reduction of ferricyanide and indophenol dye, one can say only that some pathway not involving lipoate is available, or that the lipoate is protected from attack by arsenite. It is known that the functional lipoate is tightly bound to protein in these systems and this may in some manner make it inaccessible to the inhibitor.

Inhibition of Cholinesterases

The problem of whether the cholinesterases are SH enzymes has been much disputed inasmuch as certain SH reagents do not readily inhibit. Arsenicals do inhibit but the pattern is different from that with most enzymes (Table 6-3). The remarkable result is that arsenite is much more potent than the organic arsenicals (R. H. S. Thompson, 1947) and this is
especially notable for the cholinesterases of plasma or erythrocytes (Mounter and Whittaker, 1953). Lewisite, phenylarsenoxide, and diphenylchloroarsine are all rather weak inhibitors, while arsenite inhibits well below 1 mM. Human erythrocytic cholinesterase inhibited by arsenite can be completely reactivated by prolonged dialysis and this was taken as evidence that the reaction is not with SH groups (Markwardt, 1953 a), although it is by no means a valid conclusion. It is odd that oxophenarsine, which is only about 1/100 as potent an inhibitor as arsenite, inactivates irreversibly. The fact that the inhibition increases with pH, in contrast to what one would predict for reaction with SH groups, along with other evidence, led Mounter and Whittaker (1953) to conclude that cholinesterase contains an SH group which is abnormal in that it is strongly modified by surrounding groups, or that arsenite inhibits by electrostatically binding to the esteratic site because of the dipole nature of the As=O bond. It seems unlikely that this second explanation could account for the slow reversibility of the inhibition or even for the rather potent inhibitions observed, since one would not expect the binding to be very strong. The mechanism of inhibition of cholinesterase is thus not evident at the present time.

**Comparison of Arsenicals**

Pentavalent arsenicals generally produce much less inhibition than the corresponding trivalent compounds, as one would expect from the fact that the pentavalent arsenicals do not react with SH groups. It may well be that some of the inhibition observed with the pentavalent arsenicals arises from trivalent impurities or reduction in the enzyme preparation. This situation is clearest when one compares arsenite and arsenate, since arsenate is reduced less readily than the organic arsenicals. We have already compared the actions of the arsenoso and arsinoso compounds as related to the concept of monothiol and dithiol enzymes. There is little one can say relative to the various phenylarsenoxides, since substitution of groups on the ring sometimes increases and sometimes decreases the inhibitory potency and there seem to be no obvious rules; indeed, most of the substituted phenylarsenoxides are of comparable potency. One rather disturbing comparison is between phenylarsenoxide and its dithioglycolate acting on urease, since the latter is at least 5 times more potent (Gordon and Quastel, 1948). It is not difficult to envision a transfer of the arsenule from the thio-glycolate to a more avid enzyme SH group, but how can one account for the greater potency of the mercaptide, unless one assumes an entirely different mechanism of inhibition?

Striking differences are often observed between the actions of arsene and of the organic arsenoxydes, in general the latter being much more potent inhibitors, as may be seen by comparing the data in Table 6-3 for succinate oxidase (Barron and Singer, 1945), α-glycerophosphate dehydrogenase
(Peters et al., 1946), acetyl-CoA carboxylase (Hatch and Stumpf, 1961), maltase (Rona et al., 1922), and choline oxidase (Rothschild et al., 1954). In these cases, lewisite or phenylarsenoxide is 50–1000 times more potent than arsenite. In many cases it is difficult to accurately compare the relative potencies, since the data are not sufficient; e.g., arsenite does not inhibit trypanosomal \( \alpha \)-glycerophosphate dehydrogenase at 0.5 mM while oxophenarsine and Melarsen inhibit 38\% at 0.01 mM (Grant and Sargent, 1961), and all we can say is that the inhibitory difference must be over 100. We have already speculated that this may be due in part to the small size of the arsenite, steric interference with substrate attachment being minimal, but it is unlikely that this is a very important reason. Likewise, electrostatic repulsion by negatively charged enzymes is not tenable, because arsenite is probably not mainly ionic and this could not account for the very great differences observed. One then seeks the answer in the abilities of the arsenicals to react with SH groups, and what evidence there is indicates that arsenite reacts with simple thiols less readily than do the phenylarsenoxides, but we cannot explain easily why this is so. Although arsenite doesn’t react well with glutathione, it reacts very readily with dimercaprol (Table 6-2). It may well be that arsenite does not form a stable mercaptide with a single SH group, i.e., a complex such as \((\text{HO})_2\text{As—S—E}\) may be unstable. If arsenite and cysteine are mixed, one cannot isolate the monothioarsinite since the dithioarsinite is the immediate product; in the case of an enzyme, if two SH groups are not available, the reaction would proceed with difficulty. Such differences between arsenite and the organic arsenoxides are not observed with the so-called dithiol enzymes.

**Specificity of Arsenical Inhibition**

Can one use the monosubstituted trivalent arsenicals to block specifically the \( \alpha \)-keto acid oxidases? Evidence will be presented later (page 668) that these arsenicals can often cause the accumulation of pyruvate or \( \alpha \)-ketoglutarate without appreciably inhibiting the glycolytic pathway or the reactions involved in the metabolism of amino acids and lipids. Here we shall look only at the relative sensitivities of the various enzymes. There are certain enzymes which are generally as inhabitable as the \( \alpha \)-keto acid oxidases, but most of these would not play a very significant role in the usual metabolic studies; such sensitive enzymes are glutathione reductase, glycine reductase, thioesterases, protein thiol dehydrogenase, certain pyrophosphatases, myo-inositol oxygenase, gulonate dehydrogenase, D-amino acid oxidase, formate transacetylase, and choline oxidase. Occasionally one must take into account the possible inhibition of acetyl-CoA carboxylase or the various aldehyde dehydrogenases or oxidases. The only other cycle enzyme which might be affected is succinate dehydrogenase. It is difficult to make a universally valid statement about the relative sensi-
tivities of the α-keto acid oxidases and succinate dehydrogenase for the reason that the sensitivities of each type of enzyme vary from species to species or tissue to tissue, as reference to Table 6-3 will show. Furthermore, the particular arsenical used determines to some extent the ability to inhibit selectively. It would probably be exceptional to have conditions under which an arsenical at any concentration is able to inhibit α-keto acid oxidation over 90% without affecting succinate oxidation, but from the results reported it might be possible, e. g., in pigeon brain. One must examine the situation in the preparation being studied and preferably determine concentration-inhibition curves for the different enzymes in mitochondrial suspensions. In any case, both of these effects would reduce cycle activity and, in those instances in which both types of enzyme are well inhibited, the final result might well be a more selective block of the cycle relative to other metabolic pathways. On the other hand, the disubstituted arsenicals, such as diphenylchloroarsine, would be expected to block the cycle at the aconitase and isocitrate dehydrogenase steps, and simultaneously, to inhibit creatine kinase to approximately the same degree but there has been no work on this point. It could be that these arsenicals under certain conditions block the transfer of high-energy phosphate between creatine and ADP selectively, especially if the cycle were not involved, as under anaerobic conditions, but insufficient data are available on the spectrum of action of these disubstituted arsenicals.

A large number of enzymes have been omitted from Table 6-3. We may note that the following enzymes are not inhibited by arsenite at the concentrations (mM) given in parentheses: acetylenecarboxylate hydrase (1), adenosine-5'-phosphosulfate reductase (1), asparaginase (4), catechol oxygenase (1), cholesterol dehydrogenase (50), citrullinase (1.7), DDT dehydrochlorinase (1), dihydrofolate reductase (10), dihydroorotate dehydrogenase (10), ergothionase (1), formyltetrahydrofolate synthetase (100), fructose-6-P phosphoketolase (10), galactonolactone dehydrogenase (1), glutamine synthetase (12), glutaric semialdehyde dehydrogenase (0.33), glyoxylate reductase (5), 3-hydroxyanthranilate oxidase (1), imidazole-N-methyl transferase (1), α-ketoisocaproate decarboxylase (5), 4-ketoproline reductase (0.1), d-lactate oxidase (10), 5,10-methylenetetrahydrofolate dehydrogenase (1), nitrate reductase (20), nucleotidase (33), ornithine carbamyl transferase (10), oxalate decarboxylase (1), oxalate oxidase (5), phenylalanine 4-hydroxylase (5), porphobilinogen isomerase (1), streptococcal proteinase (10), steroid dehydrogenase (1), thiogalactoside transacetylase (5), transketolase (0.1), and D-xylose reductase (10).
ELECTRON TRANSPORT AND OXIDATIVE PHOSPHORYLATION

Several oxidizing enzyme systems are inhibited by arsenicals and the question of the site of action in the electron transport sequence will be briefly considered. The original idea that arsenite acts like cyanide was proved to be incorrect (see page 598), and it is now generally accepted that the inhibition is exerted mainly, if not entirely, on the dehydrogenase component, and more specifically at the substrate site, unless high concentrations are used. The evidence for this is of several different types. (1) Failure to inhibit significantly the more distal portions of the electron transport sequence. Thus the oxidations of cytochrome c, indophenol dyes, and p-phenylenediamine are depressed only when higher concentrations of the arsenicals are employed (Szent-Györgyi, 1930; Cohen and Gerard, 1937; Mahler et al., 1954; Aldridge and Cremer, 1955). (2) Inhibition of the reduction of various acceptors. It has been demonstrated in many systems that the inhibitions of the reduction of methylene blue, phenazines, cytochrome c, and other acceptors are quite comparable to the inhibitions exerted on the entire system (Banga et al., 1931; Das, 1937 a; Peters et al., 1946; Slater, 1949; Mackler et al., 1954; Mahler et al., 1954; Thorn, 1959). (3) Protection by substrates or inhibitors competitive with substrates. Some instances of protection have been discussed (page 648) and here we shall note only additional work on the succinate oxidase system (Barron and Singer, 1945; Bergstermann and Mangler, 1948; Stoppani and Brignone, 1957). The relative sensitivities of many dehydrogenases do not imply that all dehydrogenases are readily inhibited, some, such as the malate dehydrogenase, being quite resistant, nor that other steps in the electron transport chain cannot be inhibited when the arsenical concentration is raised sufficiently. As in all cases, attention must be paid to using the proper concentration if a selective action is desired. It should be borne in mind that trivalent arsenicals can occasionally directly reduce components of the electron transport system. Legge (1954) showed that arsenite reduces cytochrome c in certain bacteria and such an action might account for some of the stimulations observed with the arsenicals. One factor which may be involved in the susceptibility of dehydrogenases in vivo is the lipid component and the thiols which form complexes with the enzyme, as shown by Jurtshuk et al. (1963) for the β-hydroxybutyrate: NAD oxidoreductase from heart mitochondria, the system being activated by lecithin and thiols. The dimercaprol complex is sensitive to arsenite whereas the cysteine complex is not. We do not know what the natural apodehydrogenase-lipid-thiol is.

The results on oxidative phosphorylation have been variable, as is common with the SH reagents, and it is likely that the effects of the arsenicals on the P:O ratio depend not only on the preparation used but also
on the experimental conditions. Ochoa (1940, 1941) found that the P:O ratio for the oxidation of succinate by pigeon brain dispersions drops from 2.02 to 0.94 upon addition of 0.8 mM arsenite, but this was interpreted as due to the strong inhibition of the oxidation of pyruvate formed from the succinate, restricting the system to a lower efficiency of phosphorylation, rather than a direct uncoupling action. Lehninger (1949) claimed that phosphorylation accompanying the oxidation of NADH by a particulate rat liver preparation is inhibited almost completely by 9 mM arsenite without change in the $O_2$ uptake, indicating a marked uncoupling activity, and Hunter and Ford (1955) reported a fall in the P:O ratio from 2.9 to 0 caused by 2 mM arsenite in rat liver mitochondria oxidizing $\beta$-hydroxybutyrate, but the $O_2$ uptake was inhibited 96% so that no conclusions can be drawn from this. More recently, Fluharty and Sanadi (1960, 1961) found moderate reductions in the P:O ratios for the oxidation of succinate and $\beta$-hydroxybutyrate by rat liver mitochondria produced by 1–2 mM arsenite, but it is doubtful if these results can be taken to indicate direct uncoupling. Others have observed no significant changes in the P:O ratio. Crane and Lipmann (1953) reported that $O_2$ uptake and phosphorylation are depressed equally by 0.1 mM arsenite when succinate is being oxidized by rat liver homogenate, and Aldridge (1957) could detect no change in the P:O ratio from concentrations of phenylarsenoide which depress 76% the oxidation of pyruvate by rat liver mitochondria. Fletcher and Sanadi (1962) found very little or no reduction in the P:O ratios brought about by 0.1 mM arsenite in heart mitochondria oxidizing pyruvate, $\beta$-hydroxybutyrate, and glutamate. Evidence from cellular preparations generally supports a lack of uncoupling activity for the arsenicals. For example, Stickland (1956 a) remarked that 1 mM arsenite actually stimulates somewhat the uptake of phosphate by yeast while having little effect on the respiration, although such results are difficult to interpret. It is probably fair to say that the arsenicals are generally not direct uncouplers and seldom exert this effect under ordinary conditions. This is confirmed by the lack of effect on ATP:P$^{32}$ exchange catalyzed by mitochondria, arsenite concentrations of 1–10 mM having variable but minimal actions (Plaut, 1957; Avi-Dor and Gonda, 1959; Sagisaka and Shimura, 1962 b). However, Fluharty and Sanadi (1960, 1961) have found that this exchange in rat liver mitochondria is depressed 50% by 0.05 mM arsenite. The reasons for the discrepancies are not apparent, but Swanson (1955, 1956 a, b) emphasized the importance of tonicity, various ions, and other experimental factors in determining the response of ATP:P$^{32}$ exchange to arsenite. Of course, in certain instances these exchange reactions are probably not related to oxidative phosphorylation. Enzymes catalyzing phosphate transfers, such as the kinases, are only moderately sensitive to the arsenicals (Table 6-3), and actions on them probably do not contribute much to the effects of the arsenicals on metabolism or function.
Effects of Combinations of Arsenicals and Thiols

Recent investigations by Sanadi and his collaborators have cast new light on the actions of the arsenicals on electron transport and phosphorylation. The effects of arsenicals on enzymes, metabolism, function, and growth had almost invariably been found to be prevented or reversed by dithiols such as dimercaprol (BAL), and this has led to the clinical use of BAL in arsenical poisoning. However, certain processes in liver and heart mitochondria are altered much more potently by equimolar mixtures of arsenite and BAL (i.e., by the arsenite-BAL complex) than by arsenite alone. Thus arsenite-BAL is a much more effective uncoupler than arsenite in many systems (Fluharty and Sanadi, 1960, 1961); it stimulates mitochondrial ATPase activity more (Fluharty and Sanadi, 1960), inhibits the ATP:Pi exchange more readily (Fluharty and Sanadi, 1961), and causes greater mitochondrial swelling (Fluharty and Sanadi, 1962 b). The effects on oxidative phosphorylation are particularly well seen in the work of Fletcher and Sanadi (1962) on beef heart mitochondria oxidizing various substrates (see accompanying tabulation). In these experiments

<table>
<thead>
<tr>
<th>Additions</th>
<th>Malate-pyruvate</th>
<th>β-Hydroxybutyrate</th>
<th>Glutamate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>O₂ uptake</td>
<td>P:O</td>
<td>O₂ uptake</td>
</tr>
<tr>
<td>None</td>
<td>8.6</td>
<td>2.9</td>
<td>12.2</td>
</tr>
<tr>
<td>Arsenite</td>
<td>6.5</td>
<td>2.3</td>
<td>11.2</td>
</tr>
<tr>
<td>BAL</td>
<td>9.4</td>
<td>2.7</td>
<td>12.4</td>
</tr>
<tr>
<td>Arsenite + BAL</td>
<td>4.2</td>
<td>0.5</td>
<td>5.8</td>
</tr>
</tbody>
</table>

arsenate was 0.1 mM and BAL was 0.11 mM. In some cases, as here, O₂ uptake is depressed more by arsenite-BAL than by arsenite, but in others the BAL may antagonize the inhibition by arsenite. If BAL is added in excess, the potentiating effect seen at equimolar concentrations disappears (see accompanying tabulation) (Fletcher et al., 1962). These results were
obtained with rat liver submitochondrial preparations oxidizing succinate. If the proper concentration of arsenite-BAL is used, there is often a definite stimulation of \( O_2 \) uptake, indicating a loss of respiratory control such as is caused by 2,4-dinitrophenol (Fluharty and Sanadi, 1961). BAL can be replaced by two other dithiols, dihydrolipoate and dihydrolipoamide, but all monothiols tested are ineffective with arsenite. Similar results have been obtained on myosin ATPase, arsenite-BAL stimulating 2- to 3-fold, whereas neither arsenite nor BAL alone has an effect, and here monothiols are ineffective (Fluharty and Sanadi, 1962 a).

The effects of butarsen present a different picture. This arsenical uncouples oxidative phosphorylation in liver mitochondria above 0.02 mM, but is more effective in the presence of a monothiol, while BAL reverses its action (Fluharty and Sanadi, 1963). In other words, butarsen alone acts rather like arsenite-BAL. Butarsen also activates the mitochondrial ATPase, and this is favored by some monothiols and reversed by BAL. Butarsen is able to stimulate \( O_2 \) uptake which has been depressed by oligomycin, and oligomycin inhibits the ATPase activity brought out by butarsen; thus the site of action of butarsen has been postulated to be between the electron transport chain and the oligomycin-sensitive phosphorylation coupling step.

Before considering various theories for the mechanism involved, it will be well to summarize some of the other enzymic and metabolic systems in which thiols have been shown to augment the action of the arsenicals. Some of these have been mentioned (page 645). It may be recalled that Peters and Stocken (1947) found oxophenarsine-BAL to be more toxic than oxophenarsine alone to Colpidium and rats. Various aldehyde dehydrogenases are inhibited more potently by arsenite-mercaptoethanol than by arsenite alone; indeed, the NADP-dependent yeast enzyme and the beef liver dehydrogenase are essentially unaffected by arsenite alone (Jakoby, 1958). Several other dehydrogenases do not behave in this manner. If the mercaptoethanol is replaced by BAL, any inhibition exerted by the arsenite is reversed. The incorporation of acetate into fatty acids by an enzyme preparation from rat liver is moderately inhibited by arsenite, but almost abolished when either mercaptoethanol or BAL is added (see accompanying tabulation) (Brady et al., 1960). The acetyl-CoA carboxylase

<table>
<thead>
<tr>
<th>Additions</th>
<th>Incorporation into fatty acids (( m\mu ) atoms ( C^{14} ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>28</td>
</tr>
<tr>
<td>Mercaptoethanol (6)</td>
<td>67</td>
</tr>
<tr>
<td>BAL (4.7)</td>
<td>116</td>
</tr>
<tr>
<td>Arsenite (2)</td>
<td>14</td>
</tr>
<tr>
<td>Arsenite (2) + mercaptoethanol (6)</td>
<td>0.1</td>
</tr>
<tr>
<td>Arsenite (2) + BAL (4.7)</td>
<td>1.3</td>
</tr>
</tbody>
</table>
and acyl-CoA-malonyl-CoA transcarboxylase from wheat germ respond similarly (Hatch and Stumpf, 1961). The former enzyme is inhibited 7% by 0.5 mM arsenite, 93% by 0.5 mM arsenite-BAL, and 7% when the BAL is in 4-fold excess over the arsenite. Butarsen, on the other hand, inhibits quite potently alone, and its action is only reduced by either glutathione or BAL. The synthesis of palmitate from acetyl-CoA and malonyl-CoA in pigeon liver extracts is unaffected by 0.025 mM arsenite, but inhibited 63% when mercaptoethanol is present (Bressler and Wakil, 1962). The inhibition of milk xanthine oxidase by arsenite is increased moderately by mercaptoethanol (Peters and Sanadi, 1961). Thus this phenomenon is not general but occurs sufficiently often to be of interest in interpreting how arsenicals act.

It has been assumed that a vicinal dithiol group is involved in oxidation-reduction and coupled phosphorylation, oxidation of these groups being accompanied by the formation of high-energy phosphate (Fluharty and Sanadi, 1960). It is then postulated that these groups are not easily accessible to arsenite, perhaps because of the hydrophobic nature of this region, and that the combination of the arsenite with BAL allows penetration to the dithiol site (Fluharty and Sanadi, 1961). The arsenite-BAL complex cannot very well be active by itself because excess BAL reverses the effect; furthermore, the arsenite-BAL probably does not react with a monothiol group since other SH enzymes are not inhibited. Thus a dissociation of the arsenite-BAL complex following penetration to the site is pictured, the enzyme dithiol group having roughly the same affinity as BAL for the arsenite (Fluharty and Sanadi, 1962 a). Butarsen is believed to be directly uncoupling because it can penetrate fairly well to the dithiol group, due to its greater lipid solubility, although monothiols may further increase its activity to some extent (Fluharty and Sanadi, 1963). The uncoupling by arsenite-BAL is not due to structural changes resulting from mitochondrial swelling, since kinetic and reversibility studies indicate the swelling to be secondary (Fluharty and Sanadi, 1962 b). The depression of the uncoupling action by Mg++ and NAD is not understood (Fluharty and Sanadi, 1961), but possibly these cofactors simply react at or near the vulnerable dithiol group. Although it is difficult to propose a reasonable alternative to this theory, there are facts which are not easily explained. Why are the monothiols completely ineffective in potentiating the action of arsenite? One might expect mercaptoethanol to facilitate penetration to some extent. Is arsenite really so lipid-insoluble as to need help for penetration, since it should exist mainly as arsenious acid? And, in addition, the arsenite molecule would be much smaller than the arsenite-BAL complex. Why do the monothiols often favor the activity of butarsen, while dithiols abolish its activity? The butarsen-BAL complex would be smaller and more lipid-soluble than the complexes with monothiols. There is a need for study
of other arsenicals and their complexes. With regard to the other enzymes and metabolic systems in which thiols augment arsenical action, less can be said about mechanism. It is difficult to understand why BAL should facilitate penetration of arsenite to a dithiol group on myosin ATPase, particularly since ATP, a highly ionic substance, penetrates well enough.

TRICARBOXYLATE CYCLE

The generally potent and fairly selective inhibition of the \( \alpha \)-keto acid oxidases by the arsenoso compounds should lead to definite disturbances in the operation of the cycle in particulate preparations and tissues. Oxidation of pyruvate, lactate, or acetate has been found to be strongly depressed by these arsenicals in most microorganisms and tissues (Table 6-1), as would be expected if these substances are metabolized through the cycle. The values in the table are often not accurate because correction was not made for the effects on the endogenous respiration; also the concentrations used are far too high in many cases. Nevertheless, the effectiveness of the arsenicals in blocking the oxidation of these substrates is evident. Other results on subcellular preparations oxidizing pyruvate may be found in Table 6-3. Just as certain pyruvate oxidases from microorganisms are relatively resistant to the arsenicals, the oxidation of pyruvate by some bacteria and yeasts is suppressed by arsenicals weakly or not at all. It is unlikely that the small inhibition by 5 \( \text{mM} \) arsenite in \textit{Rhodotorula} can be explained on the basis of inadequate penetration (Litchfield and Ordal, 1958). Rao and Gunsalus (1955) found that the oxidation of pyruvate by \textit{Acetobacter acetii} is insensitive to 1 \( \text{mM} \) arsenite and suggested that lipoate may not be involved.

In connection with the effects of arsenicals on pyruvate utilization, it is appropriate that we now consider the susceptibilities of other pathways of pyruvate metabolism not involving the cycle. Hellerman \textit{et al.} (1962) showed that 0.004–0.008 \( \text{mM} \) butarsen inhibits the formation of citrate from pyruvate in rat Walker carcinoma mitochondria, but has little effect on the disappearance of pyruvate. The production of \( \text{C}^{14}\text{O}_2 \) from labeled pyruvate is also unaffected by butarsen, which is in contrast to the results obtained with heart mitochondria. Possibly another pathway for pyruvate utilization not involving lipoate is involved. The anaerobic dismutation of pyruvate to acetate and lactate is generally not as well inhibited as the oxidation (Barron and Singer, 1945). Arsenite abolishes pyruvate oxidation by \textit{Lactobacillus brevis}, and the accumulation of lactate and acetoin indicates that dismutation is relatively unaffected and further that the reaction,

\[
2 \text{Pyruvate} \rightarrow \alpha\text{-acetoacetate} \rightarrow \text{acetoin}
\]
one of the commonest pathways for pyruvate in the homofermentative organisms, is not very sensitive (Walker, 1959). However, Reed et al. (1958) found the dismutation of pyruvate by extracts of *Streptococcus faecalis* to be reduced 70% by 0.1 m*M* arsenite, and the presence of lipoate during the incubation not to alter the inhibition. Furthermore, in *Asterococcus mycoides* the dismutation of pyruvate is depressed to about the same extent as oxidation (Rodwell and Rodwell, 1954 a). Thus it would be wise to withhold final judgment on the relative sensitivities of oxidation and dismutation. The condensation of pyruvate to acetoacetate seems to be well inhibited, but the condensation to acetylmethylcarbinol is more resistant (Barron and Singer, 1945). *Clostridium saccharobutyricum* ferments pyruvate to butyrate and acetate; in the presence of 1 m*M* arsenite, only acetate if formed (Cohen-Bazire et al., 1948; Cohen-Bazire and Cohen, 1949). This might imply a block of the butyrate-forming cycle which occurs in the clostridia. The fermentation of pyruvate by *Propionibacterium pentosaceum* is not affected by even 40 m*M* arsenite (Wood and Werkman, 1940). The phosphoroclastic reaction, wherein formate and acetyl-P are formed from pyruvate, is inhibited 50% by 0.01 m*M* arsenite in extracts of *Micrococcus lactilyticus* (McCormick et al., 1962). The photometabolism of pyruvate by *Rhodospirillum rubrum* is inhibited 80% by 1 m*M* arsenite (Ormerod and Elsdon, 1956). The anaerobic formation of hydrogen from pyruvate by sheep rumen microorganism LC is reduced only 22% by 2.5 m*M* arsenite and 65% by 50 m*M* arsenite (Peel, 1960). In *Aspergillus terreus* arsenite shifts the metabolism of pyruvate away from the formation of itaconate and toward the synthesis of cell material (Bentley and Thiesen, 1957). In most cases these pathways for pyruvate are not understood in detail, nor is it known if lipoate functions in these systems, but it seems adequately demonstrated that arsenite can, especially in the microorganisms, quite markedly alter the pattern of pyruvate utilization.

The effects of arsenicals on acetate metabolism are less well understood but it is evident from Table 6-4 that in most cases the utilization of acetate is potently inhibited. The acetate-activating enzyme system and various reactions forming or metabolizing acetyl-P or acetyl-AMP appear to involve lipoate — indeed, acetyl-dihydrolipoate has been demonstrated, and thus the site of the arsenical action could well be the free or bound lipoate. Goldschmidt *et al.* (1956) studied the effects of arsenite on the metabolism of labeled acetate in *Penicillium* but a concentration of 15 m*M* was used so that little can be concluded from their results. The pattern of citrate labeling is altered by arsenite, but this is undoubtedly due to effects on the operation of the cycle rather than on reactions specific for acetate. The inhibition of lactate metabolism by arsenicals is most likely attributable to its utilization through pyruvate. Lactate dehydrogenase and other enzymes attacking lactate do not appear to be very sensitive.
<table>
<thead>
<tr>
<th>Substrate</th>
<th>Organism or tissue</th>
<th>Arsenical</th>
<th>Concentration (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate</td>
<td>Neisseria gonorrhoeae</td>
<td>$m$-NH$_2$-q-AsO</td>
<td>0.1</td>
<td>94</td>
<td>Barron and Singer (1945)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-COOH-q-AsO</td>
<td>0.11</td>
<td>100</td>
<td>Stedman and Kravitz (1955)</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>Arsenite</td>
<td>33</td>
<td>100</td>
<td>Walker (1959)</td>
</tr>
<tr>
<td></td>
<td>Lactobacillus brevis</td>
<td>Arsenite</td>
<td>10</td>
<td>97</td>
<td>Edson and Hunter (1947)</td>
</tr>
<tr>
<td></td>
<td>Mycobacterium phlei</td>
<td>Arsenite</td>
<td>1.5</td>
<td>92</td>
<td>G.J.E. Hunter (1953)</td>
</tr>
<tr>
<td></td>
<td>Mycobacterium smegmatis</td>
<td>Arsenite</td>
<td>5</td>
<td>100</td>
<td>Rodwell and Rodwell (1954 a)</td>
</tr>
<tr>
<td></td>
<td>Asterococcus mycoides</td>
<td>Arsenite</td>
<td>0.05</td>
<td>16</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>0.1</td>
<td>31</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>0.25</td>
<td>47</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>0.5</td>
<td>76</td>
<td></td>
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<td></td>
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<td></td>
<td>1</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Rhodotorula gracilis</td>
<td>Arsenite</td>
<td>5</td>
<td>29</td>
<td>Litchfield and Ordal (1958)</td>
</tr>
<tr>
<td></td>
<td>Penicillium chrysogenum</td>
<td>Arsenite</td>
<td>10</td>
<td>65</td>
<td>Hockenhull et al. (1954 b)</td>
</tr>
<tr>
<td>Yeast</td>
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<td>0.25</td>
<td>20</td>
<td>56</td>
<td>Pickett and Clifton (1943)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td></td>
<td>66</td>
<td></td>
</tr>
<tr>
<td>Chicken liver slices</td>
<td>$p$-COOH-q-AsO</td>
<td>0.1</td>
<td>100</td>
<td></td>
<td>Barron and Singer (1945)</td>
</tr>
<tr>
<td>Pigeon brain dispersion</td>
<td>Arsenite</td>
<td>0.8</td>
<td>100</td>
<td></td>
<td>Ochoa (1941)</td>
</tr>
<tr>
<td>Mouse stomach</td>
<td>Arsenite</td>
<td>0.34</td>
<td>68</td>
<td></td>
<td>Davenport and Jensen (1949)</td>
</tr>
<tr>
<td>Rabbit leucocytes</td>
<td>Arsenite</td>
<td>1</td>
<td>91</td>
<td></td>
<td>Coxon and Robinson (1956)</td>
</tr>
<tr>
<td>Rat liver slices</td>
<td>$p$-NH$_2$-q-AsO</td>
<td>0.5</td>
<td>75</td>
<td></td>
<td>Barron and Singer (1945)</td>
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<tr>
<td>Sample Type</td>
<td>Compound</td>
<td>Amount</td>
<td>Reference</td>
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<tr>
<td>Rat liver mitochondria</td>
<td>$\varphi$-AsO</td>
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<td>47</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>0.0002</td>
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<td>Rat kidney slices</td>
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<td></td>
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<td>Arsenite</td>
<td>0.1</td>
<td>96</td>
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<tr>
<td>Rat brain suspension</td>
<td>Oxophenarsine</td>
<td>0.84</td>
<td>86</td>
<td></td>
<td></td>
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<tr>
<td>Rat brain slices</td>
<td>Oxophenarsine</td>
<td>0.84</td>
<td>60</td>
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<td>Acetate</td>
<td><em>Brucella abortus</em></td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>100</td>
<td>Altenbern and Housewright (1952)</td>
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<td></td>
<td><em>Staphylococcus aureus</em></td>
<td>Arsenite</td>
<td>33</td>
<td>100</td>
<td>Stedman and Kravitz (1955)</td>
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<td></td>
<td><em>Corynebacterium creatinovorans</em></td>
<td>$m$-$\text{NH}_2$-$\varphi$-AsO</td>
<td>0.2</td>
<td>98</td>
<td>Singer and Barron (1945)</td>
</tr>
<tr>
<td></td>
<td><em>Mycobacterium phlei</em></td>
<td>Arsenite</td>
<td>1.5</td>
<td>100</td>
<td>Edson and Hunter (1947)</td>
</tr>
<tr>
<td></td>
<td><em>Penicillium chrysogenum</em></td>
<td>Arsenite</td>
<td>10</td>
<td>100</td>
<td>Hockenhull et al. (1954 b)</td>
</tr>
<tr>
<td></td>
<td>Yeast</td>
<td>$\varphi$-COOH-$\varphi$-AsO</td>
<td>0.5</td>
<td>21</td>
<td>Singer and Barron (1945)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>97</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td><em>Chlorella vulgaris</em></td>
<td>Arsenite</td>
<td>4</td>
<td>100</td>
<td>Merrett and Syrett (1960)</td>
</tr>
<tr>
<td></td>
<td><em>Paramecium caudatum</em></td>
<td>Arsenite</td>
<td>0.1</td>
<td>90</td>
<td>Holland and Humphrey (1953)</td>
</tr>
<tr>
<td></td>
<td>Guinea pig kidney suspension</td>
<td>Arsenite</td>
<td>2.5</td>
<td>88</td>
<td>Terner (1955)</td>
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<td></td>
<td>Rabbit heart mince</td>
<td>Arsenite</td>
<td>0.5</td>
<td>65</td>
<td>Barron et al. (1953)</td>
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<td></td>
<td></td>
<td>1</td>
<td>73</td>
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<td>Lactate</td>
<td><em>Mycobacterium phlei</em></td>
<td>Arsenite</td>
<td>1.5</td>
<td>18</td>
<td>Edson and Hunter (1947)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>43</td>
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<td></td>
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<td></td>
<td><em>Penicillium chrysogenum</em></td>
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<td>10</td>
<td>90</td>
<td>Hockenhull et al. (1954 b)</td>
</tr>
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<td></td>
<td>Rat brain suspensions</td>
<td>Oxophenarsine</td>
<td>0.28</td>
<td>86</td>
<td>Gordon and Quastel (1948)</td>
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<td></td>
<td></td>
<td>0.56</td>
<td>98</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Rat brain brei</td>
<td>$\varphi$-AsO</td>
<td>0.0071</td>
<td>15</td>
<td>Aldridge and Cremer (1955)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.021</td>
<td>24</td>
<td></td>
<td></td>
</tr>
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<td></td>
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<td>0.05</td>
<td>41</td>
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<tr>
<td></td>
<td></td>
<td>0.0099</td>
<td>35</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>0.03</td>
<td>87</td>
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</table>
It is interesting that arsenite is able to depress the oxidations of pyruvate and acetate *in vivo*. When arsenite is administered intraperitoneally at a dose of 5 mg/kg/day for 3–4 weeks, rats suffer some toxic effects but are not killed, and their tissues exhibit impaired metabolism (see accompanying tabulation) (Mookerjea and Sadhu, 1955). It is not known whether there is a direct effect on succinate oxidation of whether this small inhibition observed reflects inhibition of a later step. It is rather remarkable that inhibitions of this degree can exist in the tissues of living animals and one would like to know more of the metabolic patterns in these poisoned animals. It may also be noted that when lewisite is applied to the skin of rats at a dose of 45–95 μg/g skin, the oxidation of pyruvate is inhibited 58% while the oxidation of succinate is scarcely altered, and doses of 125–160 μg/g completely block pyruvate oxidation (Thompson, 1946).

The oxidations of the cycle intermediates are inhibited to varying degrees by the arsenicals. Comparative studies are uncommon (see accompanying tabulation). Arsenite was 1 mM in both cases. Essentially the same relative susceptibilities are seen for avocado (Avron and Biale, 1957) and the fungus (J. G. H. Wessels, 1959). The difference in the patterns of inhibition by mono- and disubstituted arsenicals is well illustrated in the results

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% Inhibition of oxidation of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pyruvate</td>
</tr>
<tr>
<td>Liver slices</td>
<td>—</td>
</tr>
<tr>
<td>Kidney slices</td>
<td>40</td>
</tr>
<tr>
<td>Brain brei</td>
<td>68</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substrate</th>
<th>% Inhibition in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Avocado particles</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>94</td>
</tr>
<tr>
<td>Citrate</td>
<td>55</td>
</tr>
<tr>
<td><em>cis</em>-Aconitate</td>
<td>52</td>
</tr>
<tr>
<td><em>α</em>-Ketoglutarate</td>
<td>100</td>
</tr>
<tr>
<td>Succinate</td>
<td>25</td>
</tr>
<tr>
<td>Fumarate</td>
<td>81</td>
</tr>
<tr>
<td>Malate</td>
<td>84</td>
</tr>
</tbody>
</table>
of Aldridge and Cremer (1955) on rat liver mitochondria (see accompanying tabulation), in which the concentrations required to inhibit 50% were

<table>
<thead>
<tr>
<th>Substrate</th>
<th>p\textsubscript{I}_{50} (\psi)-AsO</th>
<th>p\textsubscript{I}_{50} (\text{di}\psi)-AsCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate</td>
<td>6.50</td>
<td>4.90</td>
</tr>
<tr>
<td>Citrate</td>
<td>5.65</td>
<td>5.35</td>
</tr>
<tr>
<td>(\alpha)-Ketoglutarate</td>
<td>6.10</td>
<td>5.05</td>
</tr>
<tr>
<td>Succinate</td>
<td>&lt;4.5</td>
<td>&lt;4.2</td>
</tr>
<tr>
<td>Malate</td>
<td>6.25</td>
<td>4.95</td>
</tr>
</tbody>
</table>

determined. In all of this work one must realize that more than the direct effect on the enzyme acting on the substrate is measured; e. g., the inhibition of malate oxidation is probably not due to an action on the malate dehydrogenase.

The effects of arsenite on the utilization of citrate are variable and depend on the preparation and the experimental conditions. The oxidation of citrate is sometimes moderately inhibited, as in avocado or \textit{Schizophyllum} (see above), but is often rather resistant. Krebs and Johnson (1937) reported that the oxidation of citrate by pigeon muscle mince is not affected by 3 mM arsenite, although the oxidation is made incomplete, presumably by blocking at the \(\alpha\)-ketoglutarate oxidase, and similar results have been obtained in yeast (Hirsch, 1952), mycobacteria (Yamamura \textit{et al.}, 1954), rat kidney (E. H. Kaplan \textit{et al.}, 1954), and rat liver (Sherman and Corley, 1952). On the other hand, citrate oxidation is completely inhibited by 5 mM arsenite in epiphyseal cartilage (Whitehead and Weidmann, 1959), and lewisite oxide at 0.0033 mM inhibits isocitrate oxidation 92% in rat liver mitochondria (Chappell, 1964 a). Chappell believes that this latter inhibition is not direct but is due to the block of malate formation and a deficiency of NADP, the regeneration of which is mediated through malate dehydrogenase and a transhydrogenase. The oxidation of isocitrate in rat brain mitochondria is not affected by 10 mM arsenite when NADP is added (Murthy and Rappoport, 1963). These results demonstrate the complex interrelationships which must be taken into account in interpreting the effects of the arsenicals on the cycle. Anaerobic citrate dissimilation by \textit{Aerobacter} is readily inhibited by arsenite around 3 mM (Brewer and Werkman, 1939; Dagley and Dawes, 1953). The formation of citrate is, of course, quite strongly inhibited in all the organisms and tissues studied.
ACCUMULATION OF KETO ACIDS AND OTHER SUBSTANCES

The inhibition of keto acid oxidation should under certain circumstances lead to the accumulation of the keto acids, and this has been found to be the case in a variety of organisms and tissues (Table 6-5). These results can perhaps be taken as the best evidence that a relatively selective inhibition can be exerted by the arsenicals. The keto acid levels often rise very markedly; sometimes no pyruvate can be detected in the controls while it progressively accumulates in the inhibited preparations to quite high levels, e.g., in *Microsporum canis* (Chattaway et al., 1956) and *Bacillus megaterium* (Duff and Webley, 1958). The pattern and degree of accumulation will depend on several factors, particularly on the pathways by which the keto acids are formed, the pathways by which they are metabolized, and the relative effects of the arsenical on these pathways.

Let us first consider a simple pathway such as

\[
\text{glutamate} \rightarrow \alpha\text{-ketoglutarate} \rightarrow \text{succinate}
\]

(or a comparable system for alanine). The effect of an arsenical on \(\alpha\)-ketoglutarate concentration will depend on the relative inhibitions on the two reactions. Since glutamate dehydrogenase and transaminases are moderately sensitive to the arsenicals (Table 6-3), one would expect, as the arsenical concentration is increased, to find first a rise in the level of \(\alpha\)-ketoglutarate and then a fall, so that if too high a concentration of arsenical is used, no \(\alpha\)-ketoglutarate would be formed. Thus one must exert care in choosing the proper concentration of arsenical if marked accumulation is to be demonstrated. This will, of course, apply to any pathway of keto acid metabolism. If we now turn to the usual glycolytic formation of pyruvate and its oxidation through the cycle, represented schematically by

\[
\text{Glucose} \rightarrow x_1 \rightarrow x_2 \rightarrow ... \rightarrow \text{pyruvate} \rightarrow \text{CO}_2 + \text{H}_2\text{O}
\]

the arsenicals will induce accumulation of pyruvate only if they act more strongly on the entry of pyruvate into the cycle than on the EM pathway. Since there are numerous instances in which pyruvate levels are increased with glucose as the substrate, it is evident that arsenicals can depress the utilization of pyruvate more than its formation. Nevertheless, since some of the glycolytic enzymes are inhibitable by the arsenicals, the concentration should be chosen with care, and preferably several concentrations tested, a procedure which has actually been used very seldom. The degree of pyruvate accumulation will also depend on the rate at which it is formed and hence on the choice of the substrate. Chattaway et al. (1956) showed that much pyruvate was formed from glucose (78), less from fructose, mannose,
**Table 6-5**

**Accumulation of Keto Acids During Arsenical Inhibition**

<table>
<thead>
<tr>
<th>Organism or tissue</th>
<th>Substrate</th>
<th>Arsenical</th>
<th>Keto acid&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacteria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aerobacter aerogenes</em></td>
<td>Citrate</td>
<td>Arsenite</td>
<td>P</td>
<td>Dagley and Dawes (1953)</td>
</tr>
<tr>
<td></td>
<td>Glucose</td>
<td>Arsenite</td>
<td>K, P</td>
<td>Fowler and Werkman (1955)</td>
</tr>
<tr>
<td><em>Azotobacter vinelandii</em></td>
<td>Sucrose</td>
<td>Arsenite</td>
<td>P</td>
<td>Dilworth (1962)</td>
</tr>
<tr>
<td><em>Bacillus megaterium</em></td>
<td>Glucose</td>
<td>Arsenite</td>
<td>P</td>
<td>Duff and Webley (1958)</td>
</tr>
<tr>
<td><em>Brucella abortus</em></td>
<td>Fumarate</td>
<td>Arsenite</td>
<td>P</td>
<td>Altenbern and Housewright (1951)</td>
</tr>
<tr>
<td></td>
<td>Citrate</td>
<td>Arsenite</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Glucose</td>
<td>Arsenite</td>
<td>P</td>
<td>Dawes and Foster (1956)</td>
</tr>
<tr>
<td><em>Mycobacterium sp.</em></td>
<td>Glycerol</td>
<td>Arsenite</td>
<td>P</td>
<td>G.J.E. Hunter (1953)</td>
</tr>
<tr>
<td><em>Mycobacterium BCG</em></td>
<td>Inositol</td>
<td>Arsenite</td>
<td>K, P</td>
<td>Ottey and Bernheim (1956)</td>
</tr>
<tr>
<td><em>Mycobacterium phlei</em></td>
<td>Glucose</td>
<td>Arsenite</td>
<td>P</td>
<td>Andrejew (1954)</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>Glucose</td>
<td>Arsenite</td>
<td>P</td>
<td>Andrejew (1954)</td>
</tr>
<tr>
<td><em>Neisseria gonorrheae</em></td>
<td>Glutamate</td>
<td>Arsenite</td>
<td>K</td>
<td>Tonhazy and Pelczar (1953)</td>
</tr>
<tr>
<td><em>Nocardia erythropolis</em></td>
<td>p-Nitrobenzoate</td>
<td>Arsenite</td>
<td>P, O</td>
<td>Cartwright and Cain (1959)</td>
</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
<td>Glucose</td>
<td>Arsenite</td>
<td>P</td>
<td>Lewis <em>et al.</em> (1955)</td>
</tr>
<tr>
<td><em>Pseudomonas saccharophila</em></td>
<td>Glucose</td>
<td>Arsenite</td>
<td>P</td>
<td>Entner and Doudoroff (1952)</td>
</tr>
<tr>
<td></td>
<td>Arabinose</td>
<td>Arsenite</td>
<td>K</td>
<td>Weimberg and Doudoroff (1955)</td>
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<tr>
<td><em>Rhodospirillum rubrum</em></td>
<td>Succinate</td>
<td>Arsenite</td>
<td>K</td>
<td>Ormerd and Elsdon (1956)</td>
</tr>
<tr>
<td><em>Sarcina lutea</em></td>
<td>Glucose</td>
<td>Arsenite</td>
<td>P</td>
<td>Dawes and Holms (1957)</td>
</tr>
<tr>
<td>Organism or tissue</td>
<td>Substrate</td>
<td>Arsenical</td>
<td>Keto acid&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Reference</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------</td>
<td>-----------</td>
<td>-----------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>Glucose</td>
<td>Arsenite</td>
<td>K, P</td>
<td>Wasserman and Hopkins (1958)</td>
</tr>
<tr>
<td><em>Shigella flexneri</em></td>
<td>Glutamate</td>
<td>Arsenite</td>
<td>K</td>
<td>Yee et al. (1958)</td>
</tr>
<tr>
<td><em>Streptococcus faecalis</em></td>
<td>Gluconate</td>
<td>Arsenite</td>
<td>P</td>
<td>Sokatch and Gunsalus (1957)</td>
</tr>
</tbody>
</table>

**Fungi**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate</th>
<th>Arsenical</th>
<th>Keto acid&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus niger</em></td>
<td>Glucose</td>
<td>Arsenite</td>
<td>K, P</td>
<td>Walker et al. (1951)</td>
</tr>
<tr>
<td><em>Aspergillus terreus</em></td>
<td>Glucose</td>
<td>Arsenite</td>
<td>P</td>
<td>Eimhjellen and Larsen (1955)</td>
</tr>
<tr>
<td><em>Itaconate</em></td>
<td>Arsenite</td>
<td>Itaconate</td>
<td>P</td>
<td>Shimi and Nour El Dein (1962)</td>
</tr>
<tr>
<td><em>Microsporum audouini</em></td>
<td>Glucose</td>
<td>Arsenite</td>
<td>K, P</td>
<td>Chattaway et al. (1956)</td>
</tr>
<tr>
<td><em>Penicillium chrysogenum</em></td>
<td>Lactate</td>
<td>Arsenite</td>
<td>K</td>
<td>Hockenhull et al. (1951)</td>
</tr>
<tr>
<td><em>Schizophyllum commune</em></td>
<td>Citrate</td>
<td>Arsenite</td>
<td>K</td>
<td>J.C.H. Wessels (1959)</td>
</tr>
<tr>
<td><em>Streptomyces coelicolor</em></td>
<td>Glucose</td>
<td>Arsenite</td>
<td>P</td>
<td>Cochrane (1952)</td>
</tr>
</tbody>
</table>

**Yeast**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate</th>
<th>Arsenical</th>
<th>Keto acid&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida krusei</em></td>
<td>Glucose</td>
<td>Arsenite</td>
<td>P</td>
<td>Ramachandran and Walker (1957)</td>
</tr>
</tbody>
</table>

**Plants**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate</th>
<th>Arsenical</th>
<th>Keto acid&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ochromonas malhamensis</em></td>
<td>Glucose</td>
<td>Arsenite</td>
<td>P</td>
<td>Reazin (1956)</td>
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<td>(alga)</td>
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</tr>
<tr>
<td><em>Prototheca zooph</em></td>
<td>Acetate</td>
<td>Arsenite</td>
<td>K, P</td>
<td>Calley and Lloyd (1964)</td>
</tr>
<tr>
<td>(alga)</td>
<td></td>
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</tr>
<tr>
<td><em>Avocado particles</em></td>
<td>Citrate</td>
<td>Arsenite</td>
<td>K</td>
<td>Avron and Biale (1957)</td>
</tr>
<tr>
<td>Invertebrates</td>
<td>Vertebrates</td>
<td>Guinea pig kidney</td>
<td>Rat heart (in vivo)</td>
<td>Rat brain</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>-----------</td>
</tr>
<tr>
<td><em>Talpaeodes eques</em> (toad)</td>
<td>Pigeon muscle</td>
<td>Mouse stomach</td>
<td>Guinea pig kidney</td>
<td>Rat heart (in vivo)</td>
</tr>
<tr>
<td><em>Eisenia fetida</em> (worm)</td>
<td><em>Lactobacillus delbrueckii</em> (lactic acid)</td>
<td>Glucose</td>
<td>Glutamate</td>
<td>Lactate</td>
</tr>
<tr>
<td><em>Trichoscyphus pulchellus</em> (fungus)</td>
<td><em>Lactobacillus delbrueckii</em> (lactic acid)</td>
<td>Malate</td>
<td>Aspartate</td>
<td>None</td>
</tr>
<tr>
<td><em>Trichoscyphus pulchellus</em> (fungus)</td>
<td><em>Lactobacillus delbrueckii</em> (lactic acid)</td>
<td>Oxophenarsine</td>
<td>Aspartate</td>
<td>Aspartate</td>
</tr>
<tr>
<td><em>Trichoscyphus pulchellus</em> (fungus)</td>
<td><em>Lactobacillus delbrueckii</em> (lactic acid)</td>
<td>Arsenite</td>
<td>Aspartate</td>
<td>Arsenite</td>
</tr>
<tr>
<td><em>Trichoscyphus pulchellus</em> (fungus)</td>
<td><em>Lactobacillus delbrueckii</em> (lactic acid)</td>
<td>Arsenite</td>
<td>Aspartate</td>
<td>Arsenite</td>
</tr>
<tr>
<td><em>Trichoscyphus pulchellus</em> (fungus)</td>
<td><em>Lactobacillus delbrueckii</em> (lactic acid)</td>
<td>Aspartate</td>
<td>Aspartate</td>
<td>Arsenite</td>
</tr>
<tr>
<td><em>Trichoscyphus pulchellus</em> (fungus)</td>
<td><em>Lactobacillus delbrueckii</em> (lactic acid)</td>
<td>Arsenite</td>
<td>Aspartate</td>
<td>Arsenite</td>
</tr>
<tr>
<td><em>Trichoscyphus pulchellus</em> (fungus)</td>
<td><em>Lactobacillus delbrueckii</em> (lactic acid)</td>
<td>Aspartate</td>
<td>Aspartate</td>
<td>Arsenite</td>
</tr>
<tr>
<td><em>Trichoscyphus pulchellus</em> (fungus)</td>
<td><em>Lactobacillus delbrueckii</em> (lactic acid)</td>
<td>Arsenite</td>
<td>Aspartate</td>
<td>Arsenite</td>
</tr>
</tbody>
</table>

* The keto acids are designated as follows: K = α-ketoglutarate, P = pyruvate, and O = oxaloacetate.
and maltose (11-24), little with galactose or lactose (3-5), and none from sucrose, lactate, succinate, or citrate in *Microsporum canis* in the presence of 5 mM arsenite.

The presence of arsénical-resistant pathways for the metabolism of the keto acids will reduce the degree of accumulation. Microorganisms especially frequently possess means of metabolizing pyruvate through reactions less sensitive than pyruvate oxidase to the arsénicals, and here some of the products of these pathways may accumulate rather than pyruvate. Some of the pyruvate will be reduced to lactate in most preparations, and the transamination of the keto acids to amino acids will often suppress their accumulation. Thus amino acids occasionally accumulate, as shown in *Aerobacter aerogenes* (Fowler and Werkman, 1955) and *Brucella abortus* (Altenbern and Housewright, 1951). Such losses of the keto acids may be very significant in balance studies. It is interesting in retrospect to consider the early observations of increased lactate following arsenite. Meyer (1881) reported that administration of arsenite to rabbits led to the appearance of some acid in the blood, and Araki (1893) and Heffter (1893) established that lactate does indeed appear in the urine and the muscles during poisoning. Morishima (1900 b) extended these observations to show that lactate also appears in the liver, kidney, and gastrointestinal tract. Accumulation of lactate has more recently been demonstrated in fibroblast cultures (Meier, 1933) and in homogenates of mammary gland and brain (Terner, 1954). It is likely that the lactate arises from pyruvate which cannot be oxidized, but originally it was thought that arsenite stimulated glycolysis (which is true if glycolysis is defined as the formation of lactate, but false if this is interpreted as an acceleration of the reactions leading to lactate). It may be noted that a Pasteur-like effect can occasionally be exerted by arsenite (see page 676).

In some instances the results with the arsénicals do not conform to the classical picture, due presumably to the special metabolic pathways present. Beevers and Gibbs (1954) tried to find the concentration of arsenite which would inhibit respiration and allow the accumulation of pyruvate in corn root tips, but found instead that ethanol and acetaldehyde accumulate, this being interpreted as a stimulation of aerobic fermentation. Xanthine is metabolized to pyruvate by *Clostridium acidi-urici* by a pathway which may be represented as

\[ \text{Xanthine} \rightarrow \text{formate} + \text{glycine} \rightarrow \text{serine} \rightarrow \text{pyruvate} \]

(Sagers and Beck, 1956). Here arsenite actually prevents the appearance of pyruvate and causes the accumulation of formate and glycine, it being in this case more inhibitory to the reaction forming serine than to pyruvate utilization. *Pseudomonas fragi* grown with D-arabinose responds normally to arsenite in that α-ketoglutarate accumulates, but cells grown with
L-arabinose are unable in the presence of arsenite to metabolize any of the pentonic acids, which are intermediates in the utilization of pentoses, and, since this block is more potently exerted than the inhibition of keto acid oxidation, no α-ketoglutarate can accumulate (Weimberg, 1961). In some organisms glucose is partly oxidized and partly transformed into cell material. Arsenite prevents pyruvate oxidation in *Acetobacter xylinum* but pyruvate does not accumulate since glucose is presumably diverted to form cellulose, so that in this case one might say that arsenite has shifted the pathway of glucose metabolism (Schramm et al., 1957). It is more difficult to explain the results of Cantrell (1951, 1953) on trypanosomes taken from rats treated with oxophenarsine, since glucose utilization was not significantly altered while less pyruvate was formed than in untreated trypanosomes. However, since untreated organisms formed 1.7 moles of pyruvate from each mole of glucose, it is evident that this trypanosome is unable to metabolize pyruvate readily, and thus the only effect noted would be a small depression in the rate of pyruvate formation, possibly certain glycolytic intermediates accumulating.

It is interesting to inquire whether arsenicals can ever allow a quantitative conversion of a substrate into a keto acid. Eimhjellen and Larsen (1955) performed a balance study on *Aspergillus terreus* with glucose as the substrate and found that 75% of the glucose accumulates as pyruvate and 20% as acetaldehyde in the presence of 10 mM arsenite, which is near-complete recovery. In the absence of arsenite, no pyruvate or acetaldehyde is detectable. *Pseudomonas saccharophila* converts each mole of glucose to around 1.7 moles of pyruvate in the presence of 2 mM arsenite (Entner and Doudoroff, 1952), and very similar results have been obtained with *Serratia marcescens*, an additional 0.6 mole being found in the form of α-ketogluconate, this corresponding to 95% recovery (Wasserman and Hopkins, 1958). The alga *Ochromonas malhamensis* almost completely oxidizes glucose, no lactate or pyruvate and very little ethanol being found, but, in the presence of 2.5 mM arsenite, 85% of the glucose in converted to these products (Reazin, 1956). Finally, in ox retina endogenous glucose and lactate are aerobically 34% converted to pyruvate, whereas with 4 mM arsenite the conversion is 65% (Futterman and Kinoshita, 1959). From these results it seems quite possible that, under the proper conditions and with the correct arsenical concentration, quantitative formation of pyruvate, and any substances arising directly from pyruvate, might be achieved.

Keto acids also accumulate in animals poisoned with arsenite. Oelkers and Vincke (1936) originally observed this in rabbits given 3 mg/kg potassium arsenite and then injected with oxalacetate 3 min later, both oxalacetate and pyruvate appearing in the urine whereas in normal animals the oxalacetate is completely metabolized. El Hawary and Thompson (1951) found blood pyruvate to rise about 5-fold in rats administered arsen-
ite subcutaneously, and El Hawary (1955) detected a similar rise in α-keto-
glutamate. Some elevation of the acetone and acetoacetate levels also occurs,
as would be expected if the operation of the cycle is impaired. A depression
of pyruvate utilization by the arsenite was also shown by the higher blood
pyruvate levels following intravenous injection of pyruvate; in normal
animals after 30 min the blood pyruvate was 1.8 mg% whereas in the arse-
nite-treated rats it was 10 mg%.

**GLUCOSE METABOLISM**

The utilization of glucose may be affected by the arsenicals either directly
of indirectly. We are thus concerned primarily with (a) the actions of the
arsenicals on the EM and pentose-P pathways, and (b) the occurrence of
a Pasteur-like effect to accelerate glucose uptake. It is further of interest
to determine the differential effects of the arsenicals on pre- and post-
pyruvate metabolism. It must be emphasized that the results on the re-
sponses of glucose metabolism to the arsenicals have varied a good deal
and have often been so divergent as to be beyond reasonable explanation.
Some results on anaerobic glycolysis are presented in Table 6-6, but in
most instances CO₂ formation was measured so that the results perhaps
do not always indicate the actual effect on lactate or ethanol production.
It is unfortunate that the organic arsenicals have not been examined in
greater detail. Despite some of the data in the table, it appears that the
EM pathway is not particularly sensitive to arsenite. Some of the evidence
for this may be summarized. (1) Arsenite often produces an accumulation
of pyruvate with glucose as the substrate, as discussed in the previous sec-
tion, indicating that the formation of pyruvate from glucose is a good deal
less sensitive than the utilization of pyruvate. (2) None of the enzymes of
the EM pathway has been found to be particularly susceptible to the arsen-
cals (Table 6-3). (3) The distal portion of the EM pathway between 3-phos-
phoglyceraldehyde and pyruvate has been found to be resistant to arsenite.
Thus Mortenson et al. (1955) noted that 3-phosphoglyceraldehyde is con-
verted readily to pyruvate by extracts of *Azotobacter vinelandii* in the pre-
sence of 10 mM arsenite, and White and Wang (1964) showed that 3-phos-
phoglycerate forms pyruvate in extracts of *Acetobacter xylinum* with
5 mM arsenite, although some inhibition occurs when the system is supple-
mented with ADP. (4) Almost complete inhibition of glucose respiration
without effect on anaerobic glycolysis has been observed in Jensen sarcoma
(Dresel, 1926) and rabbit blood (Coxon and Robinson, 1956). In the latter
instance, C¹⁴O₂ from labeled pyruvate is reduced 91% by 1 mM arsenite,
while that from labeled glucose is unaffected. (5) A number of studies
have demonstrated that the aerobic utilization of glucose is not affected
or only moderately affected by arsenite at concentrations of 1 mM or above.
Table 6-6

Effects of Arsenicals on Anaerobic Glycolysis

<table>
<thead>
<tr>
<th>Organism or tissue</th>
<th>Arsenical</th>
<th>Concentration (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clostridium tetani</td>
<td>Arsenite</td>
<td>1</td>
<td>86</td>
<td>Lerner and Pickett (1945)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Arsenite</td>
<td>2.5</td>
<td>100</td>
<td>Dawes and Foster (1956)</td>
</tr>
<tr>
<td>Yeast</td>
<td>Arsenite</td>
<td>5.5</td>
<td>16</td>
<td>Johannsohn (1874)</td>
</tr>
<tr>
<td></td>
<td>Arsenite</td>
<td>9.2</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arsenite</td>
<td>0.7</td>
<td>63</td>
<td>Schulz (1888)</td>
</tr>
<tr>
<td></td>
<td>Arsenite</td>
<td>1</td>
<td>66</td>
<td>Dresel (1926)</td>
</tr>
<tr>
<td></td>
<td>Arsenite</td>
<td>0.1</td>
<td>26</td>
<td>Dresel (1928)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>75</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>81</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arsenite</td>
<td>30</td>
<td>0</td>
<td>Szent-Györgyi (1930)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arsenite</td>
<td>0.1</td>
<td>44</td>
<td>Stickland (1956 b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arsenite</td>
<td>10</td>
<td>34</td>
<td>Ryley (1952)</td>
</tr>
<tr>
<td>Tetrahymena pyriformis</td>
<td>Arsenite</td>
<td>10</td>
<td>26</td>
<td>Ryley (1955 a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Strigomonas oncopelti</td>
<td>Arsenite</td>
<td>10</td>
<td>76</td>
<td>Agosin and von Brand (1953)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20</td>
<td>52</td>
<td>Buecling (1950)</td>
</tr>
<tr>
<td>Balantidium coli</td>
<td>Arsenite</td>
<td>1</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Schistosoma mansoni</td>
<td>Arsenite</td>
<td>1</td>
<td>52</td>
</tr>
<tr>
<td>Rat thymocytes</td>
<td>Arsenite</td>
<td>0.1</td>
<td>St 19</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>St 11</td>
<td></td>
</tr>
<tr>
<td>Rat liver slices</td>
<td>Arsenite</td>
<td>10</td>
<td>St 2</td>
<td>Goldschmidt and Lewin (1937)</td>
</tr>
<tr>
<td>Rat brain slices</td>
<td>Lewisite</td>
<td>0.1</td>
<td>89</td>
<td>Barron et al. (1947)</td>
</tr>
<tr>
<td>Rabbit bone marrow</td>
<td>Arsenite</td>
<td>1</td>
<td>15</td>
<td>Warren (1943)</td>
</tr>
<tr>
<td>Human erythrocytes</td>
<td>Arsenite</td>
<td>2.8</td>
<td>Some</td>
<td>Maizels (1951)</td>
</tr>
</tbody>
</table>
In a variety of organisms the utilization or oxidation of glucose has been reported to be depressed 0–30% by arsenite at 1–10 mM (Eimihjellen and Larsen, 1955; Fewster, 1956, 1958; Reazin, 1956; Ramachandran and Walker, 1957; Litchfield and Ordal, 1958; Planterose, 1961; Ramachandran and Gottlieb, 1963). (6) Stimulation of glucose utilization and glycolysis has often been observed, and this probably arises from an inhibition of pyruvate metabolism. This phenomenon will be discussed in some detail and then we shall consider the effects of arsenite on the pattern of glucose metabolism.

If arsenite depresses the operation of the cycle, inhibits respiration, and increases the ADP level, it might be expected to accelerate the utilization of glucose by a Pasteur-like effect, providing arsenite does not significantly inhibit the EM pathway directly and the 3-phosphoglyceraldehyde step is limiting the rate. It is not sufficient to show that lactate formation is increased, because this could result from the block of pyruvate oxidation, but an increase in glucose disappearance or CO₂ production can be taken as evidence. A stimulation of fermentative CO₂ formation aerobically by arsenite was shown in yeast (Brady et al., 1961) and corn root tips (Beevers and Gibbs, 1954). Arsenite is able to induce utilization of endogenous carbohydrate in yeast both aerobically and anaerobically, even at as high a concentration as 66 mM; anaerobically the carbohydrate utilized is increased from 323 to 800 μg/100 mg yeast, and simultaneously there are increases in CO₂ and ethanol formation (Brady et al., 1961). It appears from these results that arsenite does not depress the EM pathway. The endogenous glucose utilization by ox retina is elevated by 4 mM arsenite (Futterman and Kinoshita, 1959), the utilization of glucose by guinea pig brain slices is increased by 0.03 mM arsenite (Takagaki et al., 1958), and the glucose uptake by rat diaphragm is tripled by 1 mM arsenite (Randle and Smith, 1958). Although the mechanisms by which arsenite acts in these cases are not well understood, it is clear that arsenite is not inhibitory to glucose uptake or utilization.

Further support for a differential effect of arsenite on the cycle and glycolysis is provided by the elevation of the R.Q. generally observed. In yeast the R.Q. is increased from 1.04 to 1.41 by 0.5 mM arsenite and to 2.13 by 5 mM arsenite (Pickett and Clifton, 1943), in pea stem sections from 0.98 to 1.08 by 0.1 mM arsenite (Christiansen and Thimann, 1950 b), in corn root tips from 1.08 to 1.81 by 1 mM arsenite and to 2.38 by 10 mM arsenite (Beevers and Gibbs, 1954), in Ochromonas malhamensis from 1.10 to 1.32 by 2.5 mM arsenite (Reazin, 1956), and in carrot slices from 1.0 to 1.7 by 10 mM arsenite (Ap Rees and Beevers, 1960). These results are probably to be interpreted as a suppression of the oxidation of glucose simultaneously with little effect or a stimulation of glucose utilization. The C-1/C-6 ratio appears to be increased, often very markedly, by arsenite:
from 1.70 to 9.96 in *Streptomyces olivaceus* by 1 mM arsenite (Maitra and Roy, 1959), from 0.85 to 1.61 in carrot slices by 10 mM arsenite (Ap Rees and Beevers, 1960), and from 1.11 to 25 in guinea pig brain slices by 0.2 mM arsenite (Hoskin, 1960 b). This is a reflection of the relatively greater inhibition of pyruvate oxidation compared to glucose metabolism and does not necessarily imply the operation of the pentose-P pathway, although the increase in pyruvate might be expected to favor the pentose-P pathway by oxidizing NADPH. There is no evidence that arsenite inhibits the pentose-P pathway or the Entner-Doudoroff pathway (Lewis et al., 1955; Katznelson, 1958). For example, the conversion of 6-phosphogluconate via 3-phosphoglyceraldehyde to pyruvate in extracts of *Acetobacter* does not seem to be significantly altered by 2 mM arsenite. Usually the formation of $\text{C}^{14}\text{O}_2$ from glucose-$1-\text{C}^{14}$ is not inhibited by arsenite, even at 5 mM in the case of *Serratia marcescens* (Wasserman and Hopkins, 1958), but in brain slices 0.2 mM arsenite reduces it about 50% (Hoskin, 1960 b).

The shift in the pattern of glucose metabolism brought about by arsenite both aerobically and anaerobically is well illustrated in the balance studies of Stickland (1956 b), in which the divergence of glucose from oxidative to fermentative pathways in yeast is evident, the total amount of glucose used not being greatly altered.

The effects of the arsenicals on carbohydrate metabolism are complex and it is impossible to predict the over-all response without knowing in detail the metabolic pathways and their controls in the particular tissue. Even though the arsenical selectively blocks pyruvate oxidation, the changes in glucose uptake, respiration, polysaccharide synthesis, $\text{CO}_2$ production, and lactate concentration will be variable in different situations because they depend on what pathways are available, the relative activities of these pathways, the metabolic controls which may be operative, and the functional or metabolic state of the preparation. It is quite conceivable that the effects of an arsenical would be quite different in an actively functioning tissue compared to a resting tissue, due for one thing to the different demands for ATP; thus in a resting tissue arsenite might accelerate glucose uptake by a moderate rise in the ADP level, whereas in an active tissue the ATP might fall so low that phosphorylation of glucose might be impaired. One must also bear in mind the possible indirect effects of arsenicals mediated by actions on the growth or functional aspects of the cells.

**RESPIRATION**

Cellular respiration is inhibited by the arsenicals, as one would expect if cycle activity is impaired, but the effects are often less than would be predicted on the basis of the sensitivity of the keto acid oxidases. The results in Table 6-7 were selected to illustrate certain characteristics of arsen-
<table>
<thead>
<tr>
<th>Organism or tissue</th>
<th>Substrate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Arsenical</th>
<th>Concentration (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>G</td>
<td>Arsenite</td>
<td>0.2</td>
<td>26</td>
<td>Oka and Murachi (1954)</td>
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<td>38</td>
<td></td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>2</td>
<td>43</td>
<td></td>
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<td><em>Proteus vulgaris</em></td>
<td>G</td>
<td>Arsenite</td>
<td>2</td>
<td>74</td>
<td>Kandler <em>et al.</em> (1956)</td>
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<td>G</td>
<td>Arsenite</td>
<td>10</td>
<td>56</td>
<td>Shiio <em>et al.</em> (1961)</td>
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<td>E</td>
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<td>Vogler <em>et al.</em> (1942)</td>
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<td>5</td>
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<td>E</td>
<td>Arsenite</td>
<td>0.4</td>
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<td>Midwinter and Batt (1960)</td>
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<td></td>
<td></td>
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<td>13</td>
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<td></td>
<td></td>
<td>1.6</td>
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<td></td>
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<td>3.3</td>
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<td>Fulton and Spooner (1956 b)</td>
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<td>Edson and Hunter (1947)</td>
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<td><em>Allomyces macrognynus</em></td>
<td>G</td>
<td>Arsenite</td>
<td>0.1</td>
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<td>Bonner and Machlis (1957)</td>
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<td></td>
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<td>37</td>
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<td>10</td>
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<tr>
<td>Organism</td>
<td>Source</td>
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<td>Concentration (mM)</td>
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<td>Concentration (mM)</td>
<td>% Inhibition</td>
<td>Reference</td>
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<tr>
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<td>83</td>
<td>Levinsky and Sawyer (1963)</td>
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<td>46</td>
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<td>Barron <em>et al.</em> (1947)</td>
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<td>Time (min)</td>
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<td>32</td>
<td>Hughes and Levy (1949)</td>
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<td>19</td>
<td>Warren (1943)</td>
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<td>0.1</td>
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<td>Barron et al. (1947)</td>
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<td>49</td>
<td>Barron et al. (1947)</td>
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<td>38</td>
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<td>Arsenite</td>
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<td>36</td>
<td>Warren (1943)</td>
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<td>0.01</td>
<td>11</td>
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<td>G</td>
<td>Arsenite</td>
<td>0.2</td>
<td>51</td>
<td>Warren (1943)</td>
</tr>
<tr>
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<td>20</td>
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<td>Lardy and Phillips (1943 a)</td>
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<td>4</td>
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<td>Futterman and Kinoshita (1959)</td>
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<td>G</td>
<td>Arsenite</td>
<td>0.05</td>
<td>15</td>
<td>Planterose (1961)</td>
</tr>
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<td>0.1</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>1</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td>Human leucocytes</td>
<td>G</td>
<td>Arsenite</td>
<td>0.01</td>
<td>12</td>
<td>Warren (1943)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.1</td>
<td>32</td>
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*a Substrates are designated as follows: E = endogenous, G = glucose, and M = complex mixed medium.*
ical inhibition. The data are limited quantitatively by the fact that arsenical inhibition is usually progressive; most of the values are for inhibition over 1-hr periods, but in some cases the interval is not stated in the report. Various factors quite markedly modify arsenical inhibition (see page 684) and thus it is difficult to compare results from different investigations. The data in the table indicate that mammalian tissues are inhibited to approximately the same extent, their respiration being depressed 35–75% by arsenite at concentrations around 1 mM. One might tentatively write the tissues in the order of their sensitivities as

Kidney > brain > testis > muscle > liver > heart

based not only on data in the table but work on minces and other preparations, but this does not seem to correlate with any known differences in metabolic patterns. The microorganisms vary a great deal more in the response to the arsenicals but in general are fairly resistant, with the exception of the trypanosomes, which is surprising because these protozoa apparently do not readily metabolize pyruvate.

Arsenical-Resistant Respiration

Concentration-inhibition curves for cellular respiration are typically sigmoid with low slopes, often covering a 1000-fold range of concentration (Fig. 6-4). These curves are much less steep than those obtained with

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**Fig. 6-4.** Inhibition of the respiration of rat tissues by arsenite. (From Elliott and Norris, 1945.)
isolated enzyme systems. This might be due to the fact that respiration is the sum of a variety of reactions utilizing oxygen, the sensitivities to the arsenicals varying widely, but if this is so it indicates that inhibition of the keto acid oxidations is by no means the sole cause of the depression of respiration. It may also be noted in the curves in Fig. 6-1 that they tend to level off at inhibitions significantly lower than 100%, and this is well seen in several examples in Table 6-7, where progressive rise of the arsenical concentration above a certain level produces no further inhibition (e. g., Nocardia, Allomyces, yeast, corn root tips, Trichomonas, frog nerve, and guinea pig brain). In addition, one finds several instances in which high concentrations inhibit poorly (e. g., Thiobacillus, the mycobacteria, Penicillium, and carrot). Indeed, it is uncommon to find complete respiratory inhibition by an arsenical. It is, therefore, of some interest to inquire as to the nature of the arsenical-resistant respiration. (1) In some instances impermeability of the cell for the arsenical may account for a weak effect on respiration, but this will hardly explain the levelling off of the concentration-inhibition curves, nor does it seem to apply to the results with arsenite (although see page 698). (2) The oxidation of arsenite by enzymes might contribute occasionally but can scarcely be a major factor. (3) The keto acid oxidases of certain microorganisms are rather insensitive to the arsenicals, but again this would apply only rarely. (4) The respiration is partly attributable to oxidations not involving the cycle or keto acid oxidation in most cells. Such oxidations include those of fatty acids, amino acids, glucose, and glucose-6-P, these systems often being quite resistant to the arsenicals. (5) Pathways normally not significantly contributory may be utilized in the presence of arsenicals, particularly in microorganisms capable of metabolizing pyruvate in different ways. However, these are all speculations and we do not know in any case what the nature of the resistant respiration is. Generally the respiration in the presence of glucose is more sensitive than the endogenous respiration, but the difference is seldom marked and occasionally the opposite effect is observed; this might depend on whether glucose or its phosphorylated products can be directly oxidized.

The concentration-inhibition curves often exhibit a range in which respiratory stimulation occurs, and probably many more instances of this would be known if lower concentrations of the arsenicals had been used. This is characteristic of most SH reagents but the mechanism is unknown (see page II-879 where this problem is discussed in relation to the mercurials). A more unique respiratory response was noted by Graham (1946) in insect larvae, the inhibition disappearing as the arsenite concentration is raised. The results of Schmitt et al. (1934) on nerve may indicate a similar phenomenon but since the experiments were done at varying temperatures one cannot be certain. This possibly represents an injury response, arsenical-resistant oxidative processes being initiated, and since it was noted with other inhibitors is not a characteristic of the arsenicals.
Effects of Various Factors on Respiratory Inhibition

Florijn and his collaborators (1950) at Groningen felt that the selective inhibition of tumor cells by urethane and arsenite might be due to the higher metabolic rates and energy requirements of tumor cells, and thus investigated the effect of respiratory rate on the inhibitions. They varied the rate by changing the oxygen tension and adding 2,4-dinitrophenol. Certain aspects of these experiments have been previously discussed (page I-861; the results on kidney mince with 0.25 and 1.4 mM arsenite were shown in Fig. I-15-28). Huisman (1951) used lower arsenite concentrations and found stimulation of the respiration at low oxygen tensions (Fig. 6-5). As was stated previously, the situation is perhaps too complex to allow a simple interpretation, and these observations on respiratory stimulation make the problem even more difficult. It is unlikely that the relationship is simply one involving the respiratory rate, and it seems more reasonable to attribute the different degrees of inhibition to altered metabolic patterns, the fraction of the respiration dependent on the keto acid oxidases and the
cycle possibly increasing with oxygen tension. Whatever the explanation, these are excellent examples of how inhibition can vary with the experimental conditions and why the results of different investigators do not always check. The effects of glucose concentration on the inhibition of yeast respiration by 1 mM arsenite have bearing on this general problem (see accompanying tabulation) (Dresel, 1928). Here too there is at least a superficial correlation between the inhibition and the respiratory rate. Administration of thyroid increases the respiratory rates of tissues and increases the susceptibility of animals to arsenite (Hildebrandt and Nishiu, 1924) and organic arslenicals (Dybing, 1948). However, no significant differences were found in the effects of arsenite on the respiration of normal and hyperthyroid rat brain (Cohen and Gerard, 1937). The entire problem of the effects of metabolic rate on inhibition needs further investigation, since it is an important consideration in attempting to explain the differential effects of inhibitors on different tissues and tissues operating at various functional levels.

The respiration of liver slices from thiamine-deficient rats is inhibited by arsenite more strongly than that of normal liver (see accompanying tabulation) (Goldschmidt and Lewin, 1937). Addition of thiamine to

<table>
<thead>
<tr>
<th>Glucose (%)</th>
<th>% Inhibition</th>
<th>Respiratory rate</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>0.5</td>
<td>62</td>
<td>124</td>
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<tr>
<td>1</td>
<td>57</td>
<td>114</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>92</td>
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</table>

the slices (a) increased the respiration and the inhibition of normal liver, and (b) decreased the respiration and abolished the inhibition of deficient liver, results which are so odd that confirmation is required. One is tempted to relate the greater sensitivity of avitaminotic liver to the lack of thiamine-PP and consequent impairment of keto acid oxidation, but inasmuch as cyanide also inhibited the deficient tissue more strongly it might be better
to reserve judgment. There is no doubt that thiamine deficiency and arsenical poisoning cause similar metabolic and pathological changes, and quite possibly for this reason the deficiency would make tissues more susceptible to arsenicals, but one can argue that in the deficient tissue a smaller fraction of the respiration would involve the keto acid oxidases and hence that arsenical inhibition might be less than in normal tissue.

The experimental temperature is an important factor in determining the extent of respiratory inhibition by the arsenicals if the results obtained by Myers et al. (1962) are generally applicable. The arsenite concentration for 50% inhibition of rat thymocyte respiration is 1.8 mM at 25° but only 0.15 mM at 37°, a 12-fold difference in potency. It is unlikely that this is due to a more rapid reaction of the arsenite with the cell enzymes because the oxygen uptake was measured over a period of 1–2 hr. Some workers would conclude that this is another example in which the inhibition is correlated with the respiratory rate. Others would interpret the results as a shift in limiting reactions brought about by the temperature change. The complex effects of temperature on cellular inhibitions in general and respiratory inhibitions in particular have been discussed (Chapter I-15).

Microorganisms are often able to develop resistance to the arsenicals and it would be important to know if this is dependent on metabolic adaptations. Trypanosomes resistant to oxophenarsine exhibit no obvious metabolic differences with respect to substrate utilization, level of respiration, or inhibition by a variety of substances, but it requires about 10 times the concentration of oxophenarsine, relative to the normal strain, to depress the respiration 50% (Harvey, 1949). If this resistance in the trypanosomes is a matter of reduced permeability to the arsenual, for which there is good evidence (page 766), this is a good example of how respiratory inhibition can be modified, not through change in the metabolic pattern but simply by change in the degree to which the inhibitor can reach its site of action. This could easily be checked by determining whether there is alteration of the respiratory response to other arsenicals, such as arsenite, since the permeability changes are reasonably specific. Mycobacteria resistant to isoniazid do not show a modified respiratory response to arsenite (Müller et al., 1960).

Inhibition of Tissue Respiration *in Vivo*

In order to determine if respiratory inhibition occurs in the tissues following the administration of arsenicals to animals, Elliott and Norris (1945) injected rats intraperitoneally or subcutaneously with 25 mg/kg As₂O₃ (which is lethal in 15 min and 50 min, respectively) and sacrificed the animals when signs of imminent death occurred. The tissues were removed, sliced, and their respiration measured (see accompanying tabulation). The brain is quite resistant to respiratory depression, perhaps due to the slow penetration of the arsenite into this tissue, but the question arises
as to the origin of the central nervous system disturbances observed. One explanation is that only specific regions of the central nervous system are permeable to arsenite and one would not detect this in measuring the respiration of the cerebral cortex. Another explanation is that some effect other than depression of respiratory systems is involved. Indeed, one wonders if direct central actions are exerted in these acute experiments, since the behavior of the animals could be explained in other ways. The difference is response of the liver when the arsenite was given by the two routes is not easy to interpret, unless the arsenite can more readily act on the liver when administered into the abdominal cavity.

Huston and Martin (1955) injected 25 mg/kg sodium arsenite intraperitoneally and sacrificed the rats at 30-45 min. The tissues were sliced and either placed on mats suspended in a gas phase or put into media. Both Krebs-Ringer-phosphate and the Krebs medium III were used; the latter is a phosphate-bicarbonate medium with pyruvate, fumarate, and glutamate in addition to glucose (see accompanying tabulation). Changes of less than 10% were considered to be without significance. The higher inhibitions when the tissues were suspended on mats were attributed to the fact that the arsenite and thioarsenites are unable to diffuse out of the slices. Again it is evident that the respiration of the brain is unaffected; even several successive doses of arsenite, making the animals hypothermic and toxic, produced no inhibition in the brain. The different results in the two

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% Change of respiration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>− 44</td>
</tr>
<tr>
<td>Kidney cortex</td>
<td>− 14</td>
</tr>
<tr>
<td>Liver</td>
<td>− 30</td>
</tr>
<tr>
<td>Cerebral cortex</td>
<td>+ 4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% Change of respiration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mats</td>
</tr>
<tr>
<td>Diaphragm</td>
<td>− 37</td>
</tr>
<tr>
<td>Kidney</td>
<td>− 31</td>
</tr>
<tr>
<td>Liver</td>
<td>− 60</td>
</tr>
<tr>
<td>Brain</td>
<td>+ 5</td>
</tr>
<tr>
<td>Neck muscle</td>
<td>− 62</td>
</tr>
<tr>
<td>Heart</td>
<td>− 37</td>
</tr>
</tbody>
</table>
media point out once more how the experimental conditions can modify the results obtained with inhibitors, but are difficult to understand since the addition of pyruvate and glutamate would be expected to make the respiration more sensitive to arsenite. Actually, the respiratory rates in medium III were not much higher than in the KRP medium, and the highest rates were seen in the tissues respiring endogenously on the mats. In any event, these two investigations demonstrate clearly that near-lethal doses of arsenite can quite markedly disturb respiratory activity in most tissues.

**Mechanism of Respiratory Inhibition**

A relatively selective depression of keto acid oxidation by the arsenicals has been observed in many organisms and tissues, if one assumes that the accumulation of pyruvate and \( \alpha \)-ketoglutarate is valid evidence for this. It has thus been generally thought that respiratory inhibition by the arsenicals is mainly or completely due to the action on these steps in the cycle, and arsenicals have been used to demonstrate the existence of the cycle and the quantitative importance of the cycle in the total respiration. There certainly are better means of establishing the presence or absence of the cycle, but the use of the arsenicals to determine the contribution by the cycle requires further comment. This problem has been touched on previously (page 662) and it was concluded that the nature of the concentration-inhibition curves, and the high concentrations often required to inhibit respiration markedly, make it difficult to decide how much of the inhibition arises from depression of the cycle and how much is contributed from actions elsewhere of the arsenicals. If one is using these inhibitors to estimate the cycle activity, one must choose a concentration, or range of concentrations, and how is this to be done? It might be suggested that the concentration producing the maximal accumulation of keto acids be used; however, if this is determined, there is little point in measuring respiratory depression. Furthermore, one cannot be sure that even at arsenite concentrations below 1 mM there is no inhibition of enzymes other than the keto acid oxidases; for example, lipoate functions in several systems and a few other enzymes are quite sensitive (page 655). It may well be possible to find a concentration which in a particular tissue and under proper conditions will selectively interfere with the oxidation of keto acids and the operation of the cycle, but there is no way to do this without a through investigation of the tissue’s metabolism and its response to the arsenical. The results in Table 6-7 do not, I feel, reflect the cycle contribution to the respiration, particularly for those tissues, such as kidney or heart, in which the cycle is probably the major oxidative pathway.*

* Tissue preparations usually are functionally inactive and this may reduce the relative importance of the cycle.
An action of arsenite generally ignored is the lowering of the coenzyme A level. Estler et al. (1960) found that the coenzyme A in yeast is reduced 25% by 1.1 mM arsenite and 50% by 4.8 mM arsenite. Such an effect would not only interfere further with keto acid oxidation, but should suppress a variety of reactions. It is doubtful if reaction with other monothiols is very important, and it has been shown that massive doses of neoarsphenamine are required in rabbits to lower tissue glutathione levels even slightly (Brown and Kolmer, 1929). There may of course be, unknown thiols of metabolic importance with which the arsenicals readily react.

**VARIOUS METABOLIC PATHWAYS**

Inasmuch as many pathways of metabolism are intimately related to the formation and utilization of acetyl-CoA, and hence to keto acid oxidation and the operation of the cycle, the arsenicals might be expected to alter rather extensively the metabolic pattern indirectly in addition to any direct effects they might have on these pathways. It is usually difficult to distinguish between the primary and secondary effects in tissues or whole animals, and it is probable on the basis of the information we have that both always occur simultaneously.

**Lipid Metabolism**

It has been stated many times that arsenite administered in subtoxic doses over a period of time increases assimilative processes through an inhibition of oxidations, and that this can lead to an acceleration of growth and increased body weight (see page 725). At certain dosage levels there may be an increase in total body fat without significant change in the body weight (Nakazawa, 1931); higher doses depress the food intake and cause a loss of weight. Fatty degeneration of the liver during chronic arsenical poisoning has been noted frequently, but accumulation of lipid occurs in many tissues, even in cartilage cells (Tinacci, 1952) and the perfused rabbit heart (Tanzi, 1938).* The effects of the arsenicals on lipid metabolism must be very complex and no detailed study of this has yet been made. It was assumed by the early workers that arsenicals depress lipid cata-

* Fatty degeneration or infiltration of tissues has often been taken as evidence for an increased lipid content, but this is not necessarily true since histologically the fat may become more visible, due to profound changes in cytoplasmic structure, without marked alterations in total content. Thus rabbit livers exhibiting severe necrosis and fatty degeneration due to arsphenamine administration show relatively minor changes in lipid composition, there being possibly a slight rise in cholesterol and some decrease in neutral fats (MacLachlan, 1940).
bolism by inhibiting lipases, since these enzymes are sensitive in some instances, particularly to atoxyl (Table 6-3). The extremely potent inhibitions reported by Rona and his collaborators have not been confirmed by other investigators, and it is very unlikely that the arsenicals at the concentrations occurring in the tissues during poisoning exert an appreciable effect on lipid metabolism through this mechanism. However, histochemical studies must be done to settle this point.

Both the synthesis and oxidation of fatty acids are readily inhibited by the arsenicals. The incorporation of acetate into fatty acids by a soluble system from pigeon liver is completely blocked by 0.1 mM arsenite (Brady et al., 1956), but is much more resistant in chloroplasts, only 25% depression being observed with 0.5 mM (Mudd and McManus, 1964). Potent inhibition of synthesizing systems from rat liver (Brady et al., 1960) and pigeon liver (Bressler and Wakil, 1962) requires the addition of a monothiol, such as mercaptoethanol, and this with the high sensitivity to arsenicals has led Brady (1960) to postulate the presence of vicinal SH groups, although these appear not to be lipoate which is present in insignificant amounts in the pigeon liver preparation. The condensation of acetyl-CoA (or butyryl-CoA) with malonyl-CoA is not markedly inhibited so that the dithiol group may function in the subsequent reductive reaction. Turning to the oxidation of fatty acids, one finds that the conversion of hexanoate and \( \Delta^2 \)-hexenoate to acetoacetate by rat liver homogenate (Witter et al., 1950), and the oxidation of palmitate by a particulate fraction from peanut cotyledons (Humphreys et al., 1954), are strongly depressed by arsenite, the latter completely by 0.1 mM. Pea stem sections incubated with auxin convert fats into sugars, and 0.1 mM arsenite — at which concentration growth is depressed 50% — inhibits this around 90% (Christiansen and Thimann, 1950 b). The sites of inhibition and the effects of the arsenicals on the various enzymes of the fatty acid oxidation helix are unknown. It is thus clear that both the formation and utilization of the long-chain fatty acids are very sensitive to the arsenicals. On the other hand, the oxidation of butyrate, at least by locust thorax particles, is relatively unaffected (Meyer et al., 1960). Although utilization of acetoacetate by guinea pig kidney slices is fairly strongly inhibited by arsenite (Quastel and Wheatley, 1935), the synthesis of acetoacetate from acetyl-P in the presence of coenzyme A, phosphotransacetylase, and thiolase is only weakly inhibited (Drummond and Stern, 1960), but the conversion of pyruvate to acetoacetate would probably be readily blocked. Inasmuch as all the reactions forming or utilizing acetyl-CoA in the accompanying diagram are quite potently inhibited by the arsenicals, it is difficult to predict what would happen to the fatty acid levels, particularly in vivo. The relative degrees of inhibition exerted on these four reactions by a chosen concentration of arsenical would determine the over-all effect, and this would undoubtedly change with
the arsenical concentration. Of course, there are a number of other reactions forming acetyl-CoA (e.g., from the ketogenic amino acids) and utilizing acetyl-CoA (e.g., the synthesis of sterols), and these must also be taken into account.

The synthesis of cholesterol from acetate is strongly inhibited by arsenite but there is some disagreement as to the major sites of actions. The conversion of mevalonate $\rightarrow$ cholesterol is moderately sensitive, being depressed 75-90% by 1 mM arsenite (Moller and Tchen, 1961; Gaylor, 1963), as is the conversion of mevalonate $\rightarrow$ squalene. The conversion of squalene $\rightarrow$ sterols is not much affected by arsenite (Goodman, 1961; Moller and Tchen, 1961). There appear to be two sensitive sites in this portion of the sequence. One of these is on the pathway from mevalonate $\rightarrow$ squalene; the site of attack is not on the reaction of 5-P-mevalonate $\rightarrow$ 5-PP-mevalonate (Hellig and Popjak, 1961), or the reaction of farnesyl-PP $\rightarrow$ squalene (Goodman and Popjak, 1960). The other sensitive site lies between lanosterol and cholesterol. Moller and Tchen (1961) claimed that arsenite causes the accumulation of radioactivity from mevalonate-2-C$^{14}$ in lanosterol during incubation with a rat liver homogenate, whereas Gaylor (1963) found the radioactivity to appear mainly in lanosta-7,24-dien-3β-ol when rat epidermis is used, the labeling of lanosterol not being altered. Moller and Tchen (1961) believe that the most sensitive step is the oxidation of lanosterol. It is interesting that the inhibition of cholesterol formation is enhanced by equimolar dimercaprol and no reversal of the inhibition is seen with 10-fold excess dimercaprol (Gaylor, 1963). This might be taken by some as evidence for vicinal SH groups in one or more of the enzymes involved. The synthesis of corticoids by rat adrenal sections seems to be less sensitive to arsenite than is the synthesis of cholesterol, about 75% inhibition being exerted by 1 mM arsenite (Schönbaum et al., 1956). Unfortunately, there has been no conclusive work on the effects of arsenicals on the formation of mevalonate from acetate or acetyl-CoA, and it may well be that additional sensitive sites lie in this portion of the pathway. On the basis of these results one might expect to find a depression of cholesterol levels in vivo, but such is not the case. Auken (1945) reported that...
the administration of arsenite to eight human subjects for 10 days led to a mean increase of 26 mg% in free cholesterol and 44 mg% in total cholesterol in the blood, and Mookerjea and Sadhu (1955) found marked increases in both free and esterified cholesterol in the kidneys of rats given arsenite for 3–4 weeks, although the changes in the liver and blood are much less. It may well be that in these experiments the low tissue concentration of arsenite led to a diversion of two-carbon fragments to sterol synthesis as a result of the inhibition of the cycle, and that higher arsenite concentrations would have depressed the formation of the sterols.

Much less is known about the effects of arsenicals on phospholipid metabolism, but Cantrell (1951) found that trypanosomes isolated from rats treated with oxophenarsine exhibit an impaired incorporation of labeled phosphate into phospholipids; since glucose utilization is unaffected, he postulated that the arsenicals may well act primarily on lipid metabolism in these organisms. However, Mazelis and Stumpf (1955) reported that arsenite does not affect the incorporation of labeled phosphate into phospholipids in peanut mitochondria with succinate as the substrate. More work must be done in this field before conclusions can be drawn, and might be profitable in the light of the recently discovered functional significance of some of the phospholipids.

Nitrogen Metabolism

Arsenicals may also affect protein and amino acid metabolism either directly or indirectly, and the relations are so complex that the resultant changes in the nitrogenous constituents of the whole animal are difficult to interpret. The earliest workers found little effect of subtoxic doses of arsenite on protein metabolism or urinary nitrogen excretion in fasting dogs or dogs in nitrogen balance (Von Boeck, 1871; Fokker, 1872). However, Pribyl (1927) noted quite definite changes in rabbits given arsenite orally in increasing dosage over 10 days (see accompanying tabulation). It is clear that the rises in blood and urinary nitrogen are almost entirely due

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Arsenite</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPN (mg%)</td>
<td>32.1</td>
<td>40.2</td>
<td>+ 25</td>
</tr>
<tr>
<td>Urea N (mg%)</td>
<td>13.2</td>
<td>20.1</td>
<td>+ 53</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total N (mg)</td>
<td>392</td>
<td>545</td>
<td>+ 39</td>
</tr>
<tr>
<td>Urea N (mg)</td>
<td>311</td>
<td>431</td>
<td>+ 39</td>
</tr>
<tr>
<td>Ammonia N (mg)</td>
<td>4.64</td>
<td>4.92</td>
<td>+ 6</td>
</tr>
</tbody>
</table>
to urea. Cutler (1931) obtained similar results on dogs administered 75 mg/kg arsphenamine intravenously (see accompanying tabulation), the blood being obtained 25 hr later when the dogs were definitely toxic. Some of these changes are probably nonspecific in that they would occur during various types of poisoning. The elevation in the guanidine level may be the result of liver injury rather than of specific arsenical action. Since the amino acids and proteins are often in dynamic equilibria, their levels dependent on the rates of formation and utilization, a negative nitrogen balance might indicate either an inhibition of synthesis or a stimulation of breakdown.

Arsenicals should depress protein synthesis through interference with the availability of certain amino acids, by depletion of ATP, and possibly by direct actions on the synthetic pathway. The results in Table 6-8 make clear the great variation in the sensitivities in different systems. One reason for this is the different supplements used in noncellular preparations; e.g., whether the ATP is provided or must be generated by oxidative phosphorylation; in the system studied by Weissbach (1960), the incorporation of leucine was determined in a mixture of a particulate fraction, a soluble fraction, RNA, Mg++, phosphate, ATP, and a thiol. Another possible reason is that the whole sequence of protein synthesis was not measured in every investigation, since amino acids can presumably be incorporated into nonprotein fractions. It is somewhat surprising, nevertheless, that arsenite is so little inhibitory in some cases. Christiansen and Thimann (1950 c) found that amino acids disappear during incubation of pea stem sections, and that 0.1 mM arsenite depresses the utilization of the amino acids 62%, while inhibiting the formation of cell wall protein 11% and of plasma protein 71%. It is not known if arsenicals can exert a primary inhibition of protein synthesis in whole animals. Administration of arsenite to rats for 10 days led to a 27% decrease in liver arginase (Lightbody and Calvery, 1938) and it is known that arsenite poisoning in man impairs the synthesis of hemoglobin (Brugsch and Tichter, 1950), but in both instances the effect
Table 6-8

Effects of Arsenite on Amino Acid Incorporation and Protein Synthesis

<table>
<thead>
<tr>
<th>Organism or tissue</th>
<th>Process tested</th>
<th>Arsenite (mM)</th>
<th>% Inhibition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus liquefaciens</em></td>
<td>Synthesis of proteinase in nonproliferating cells</td>
<td>10</td>
<td>24</td>
<td>Rabin and Zimmerman (1956)</td>
</tr>
<tr>
<td><em>Pseudomonas</em> sp.</td>
<td>Incorporation of amino acid-C(^{14}) mixture into particulate preparation</td>
<td>0.025</td>
<td>30</td>
<td>Yoshida <em>et al.</em> (1960)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.25</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>91</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>Incorporation of leucine into noncellular preparation</td>
<td>8.3</td>
<td>9</td>
<td>Weissbach (1960)</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>Incorporation of glycine-C(^{14}) into protein</td>
<td>3</td>
<td>50</td>
<td>Stachiewicz and Quastel (1959)</td>
</tr>
<tr>
<td>Tobacco leaf discs</td>
<td>Incorporation of leucine into chloroplast protein</td>
<td>10</td>
<td>30</td>
<td>Stephenson <em>et al.</em> (1956)</td>
</tr>
<tr>
<td><em>Vigna sinensis</em></td>
<td>Incorporation of L-glutamate-C(^{14}) into mitochondrial protein</td>
<td>1</td>
<td>95</td>
<td>Das <em>et al.</em> (1964)</td>
</tr>
<tr>
<td><em>Galega officinalis</em> root nodules</td>
<td>Incorporation of valine-C(^{14}) into protein</td>
<td>0.1</td>
<td>98</td>
<td>Proctor and Moustafa (1963)</td>
</tr>
<tr>
<td>Silkworm silk gland mince</td>
<td>Incorporation of glycine-C(^{14}) into protein</td>
<td>10</td>
<td>0</td>
<td>Faulkner and Bheemeswar (1960)</td>
</tr>
<tr>
<td>Rat liver homogenate</td>
<td>Incorporation of glycine-C(^{14}) into protein</td>
<td>6</td>
<td>95</td>
<td>Peterson and Greenberg (1952)</td>
</tr>
<tr>
<td>Rabbit reticulocytes</td>
<td>Incorporation of L-leucine-C(^{14}) into protein</td>
<td>0.1</td>
<td>5</td>
<td>Borsook <em>et al.</em> (1957)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Incorporation of glycine-C(^{14}) into protein</td>
<td>1</td>
<td>90</td>
<td>Borsook <em>et al.</em> (1952)</td>
</tr>
<tr>
<td></td>
<td>Incorporation of histidine-C(^{14}) into protein</td>
<td>1</td>
<td>94</td>
<td>Borsook <em>et al.</em> (1952)</td>
</tr>
</tbody>
</table>
could be secondary. There is probably some justification for attributing at least part of the negative nitrogen balance during arsenical poisoning to interference with protein synthesis, but other factors must certainly be important.

Few enzymes involved in nitrogen metabolism are sensitive to the arsenicals (Table 6-3). Most proteolytic enzymes are resistant, except for certain cathepsins, and many of the enzymes degrading amino acids (e. g., oxidases, decarboxylases, and deaminases) are inhibited only by concentrations of the arsenicals far above those encountered in the tissues. It is true that the oxidations of some amino acids are strongly inhibited but the sites of attack are seldom the proximal enzymes. For example, it has been shown many times that the oxidation of L-glutamate is readily inhibited by arsenite, in some cases around 50% by 0.01–0.1 mM (Tonhazy and Pelczar, 1953; Kann and Mills, 1955; Shiio, 1957; Charli-Bitron and Avi-Dor, 1959 a; Das and Roy, 1961, 1962; Borst, 1962), but the site of action must be the α-ketoglutarate oxidase in most cases. Similarly, the oxidation of proline is inhibited by arsenite (Roche et al., 1953; Shiio, 1957), but the pathway proceeds through glutamate. Possibly in some tissues the glutamate dehydrogenase is a major site of inhibition as well, and arsenite has been shown to inhibit the production of ammonia by the brain (Weil-Malherbe and Green, 1955 a). This ammonia probably comes from glutamate and glutamine, but the oxygen uptake is inhibited more than the release of ammonia (Takagaki et al., 1957). The adenylate deaminase of brain is unaffected by 10 mM arsenite (Weil-Malherbe and Green, 1955 b). The transaminases are not markedly inhibited by the arsenicals, and this is further indicated by the accumulation of certain amino acids, such as alanine, aspartate, and glutamate (Altenbern and Housewright, 1951). The over-all effects of arsenicals on amino acids levels in cells will depend on many factors, including the arsenical concentration which to some extent determines the selectivity of the inhibition. It may be mentioned that essentially nothing is known of the alterations which may be produced by arsenicals in the urea cycle, but from the results on whole animals it appears that no important inhibition is exerted here.

Porphyrin Synthesis

The biosynthesis of protoporphyrin involves the conversion of glycine and succinyl-CoA to δ-aminolevulinate, followed by condensation to porphobilinogen, which further condenses to form the porphyrin nucleus (see page II-159). Arsenicals could interfere with porphyrin synthesis by decreasing the supply of either glycine or succinyl-CoA, or by inhibiting any of the reactions leading to porphyrins. Although nothing is known of the effects of the arsenicals on over-all glycine metabolism, significant depletion of glycine is unlikely in view of the many pathways forming it. However,
succinyl-CoA normally arises from α-ketoglutarate and arsenicals would potently depress this. It was shown by Lascelles (1956) in *Rhodopseudomonas spheroides*, which converts glycine and α-ketoglutarate to porphyrins and bacteriochlorophyll, that arsenite completely blocks the formation of δ-aminolevulinate at a concentration having no effect at all on the further reactions by which δ-aminolevulinate is utilized to yield coproporphyrin III, and the results of Granick (1958) also indicate a site of inhibition before δ-aminolevulinate in erythrocytes. The only distal step tested directly is the condensation of porphobilinogen to uroporphyrinogen III and it is insensitive to arsenite (Lockwood and Benson, 1960). Studies on the formation of δ-aminolevulinate in liver mitochondria likewise implicate α-ketoglutarate oxidase as a major point of arsenite attack (Granick and Urata, 1963). The effects of arsenite on the synthesis of protoporphyrin by chicken erythrocytes are shown in Fig. II-1-17. It is interesting that there is as much inhibition when glycine and succinate are used as substrates. This could result from inhibition of the formation of succinyl-CoA from succinate, but it also leaves open the possibility that arsenite inhibits δ-aminolevulinate synthetase. This could be easily determined by studying the reaction with succinyl-CoA as the substrate. The observation that arsenite causes a prolonged excretion of coproporphyrin in rabbits is probably not attributable to an effect directly on porphyrin synthesis, but to some disturbance in liver metabolism (Schwartz and Zagaria, 1951).

Photosynthesis

The photolysis of water and the evolution of oxygen are not significantly inhibited by arsenite in *Chlorella* (Kandler, 1955) or spinach chloroplasts (Arnon et al., 1956), nor is there appreciable inhibition of photophosphorylation in *Rhodospirillum rubrum* (Geller and Lipmann, 1960), chloroplasts (Arnon et al., 1956, 1959; Whatley et al., 1959), or dahlia and datura leaves (Massini, 1957). These results seem to eliminate the participation of lipoate as the primary reductant in the early photosynthetic events. Enzymes catalyzing the interconversion of NADP and NADPH in chloroplasts are likewise not inhibited (Avron and Jagendorf, 1956; San Pietro and Lang, 1958). The fixation of CO₂ by chloroplasts, however, is strongly inhibited by arsenite, as first shown by Arnon et al. (1956), the suppression being complete at a concentration without effect on the Hill reaction or photophosphorylation. This has been studied by Gibbs and Calo (1959 a, b, 1960 b), who obtained inhibitions of around 90% for the photochemical reduction of CO₂ in reconstituted chloroplast systems with 0.01 mM arsenite (whole chloroplasts are less than one tenth as sensitive). None of the enzymes involved in the conversion of CO₂ to carbohydrate is inhibited by arsenite at 1 mM, so the site for the block is unknown. The hypothetical primary photogenic substance postulated by Miyachi (1960) to play a
role in CO₂ fixation in *Chlorella* is decreased by exposure to arsenite, as well as to other SH reagents. Since ribulose-diP carboxylase is not sensitive to arsenite, possibly arsenite interferes with the generation of ribulose-diP, but the action is not on the ribulose-5-P kinase. In contrast to the evolution of oxygen, the photoproduction of hydrogen by *Rhodospirillum rubrum* is quite sensitive to arsenite and it was suggested that vicinal SH groups occur on the enzyme system (Gest et al., 1962). On the other hand, the nonphotochemical evolution of hydrogen by rumen microorganisms is inhibited poorly by arsenite (Peel, 1960), and the utilization of hydrogen for reductive and energy-supplying purposes in *E. coli* (Lascelles and Still, 1947) and *Hydrogenomonas facilis* (McFadden and Atkinson, 1957) is scarcely affected by arsenite.

**Miscellaneous Metabolic Pathways**

A few actions of arsenite on certain important metabolic systems will be mentioned only briefly because insufficient work has been done on the mechanisms involved. Luminescence of *Achromobacter fischeri* was shown to be dimmed by 3 mM arsenite and almost abolished by 4 mM arsenite (Korr, 1935), which indicates rather low sensitivity, but more recently it has been claimed that arsenite strongly inhibits the luminescence, although no concentrations were given (Rogers and McElroy, 1958). Sulfur oxidation by *Thiobacillus thiooxidans* is moderately inhibited by arsenite (28–42% by 1 mM), respiration being affected less and CO₂ fixation more (Vogler et al., 1942; Iwatsuka et al., 1962). The growth of these bacteria is stopped, however, at concentrations without effect on sulfur oxidation. The synthesis of riboflavin in the epicotyls of lupine seedlings is depressed at very low arsenite concentrations, the level of riboflavin falling 24% in the presence of 0.0001 mM arsenite, but as the concentration of arsenite is increased another action must appear because around 0.01 mM there is essentially no effect and at 0.03 mM there is an elevation of the riboflavin level (Gustafson, 1955). The deiodination of thyroxine in an extract of rat liver is unaffected by 1 mM arsenite (Maclagan and Reid, 1957). The metabolism of ethanol in rabbits is unaltered by toxic doses of arsenite, as measured by blood ethanol levels following slow intravenous infusions (Hulpié et al., 1948). Regrettably little is known about the effects of the arsencals on the synthesis of nucleotides and nucleic acids, but Skipper et al. (1951) reported that 9 mg/kg potassium arsenite injected into mice given formate-C¹⁴ quite markedly reduces visceral purines (52%) and nucleic acids (61%). Such an effect must certainly be of significance in producing the pattern of arsenite poisoning and the growth inhibitions observed. Certain other metabolic actions will be taken up in connection with functional or growth disturbances.
PENETRATION OF ARSENICALS INTO CELLS

Inasmuch as the entrance of arsenicals into cells and tissues is an important factor in determining the degree of effect produced, particularly in certain microorganisms, it will be well to take up at this time what little is known about the permeability of cells to the arsenicals. As with any substances which are bound to proteins, it is often difficult by the usual analyses to determine whether the arsenical has really entered the cells or is adsorbed or chemically reacted with extracellular material or the plasma membrane.

Arsenate

Several observations of an indirect nature purport to prove that the effects of arsenate are limited by membrane penetration. Dresel (1926) found that suspensions of avian erythrocytes and hemolysates respire at comparable rates, but that 0.05 mM arsenite inhibits the latter 40% and the former only 9%. Since the respiration was measured over 1-hr periods, it is difficult for me to believe that the differences can be attributed to permeability barriers. As discussed previously (page 602), un-ionized arsenious acid should be the dominant form in solutions at physiological pH and that it would fail to penetrate into these cells during a 1-hr period is very unlikely. Bergstermann and Mangler (1948) also reported that isolated succinate dehydrogenase is inhibited more than the enzyme in muscle brei, and thought that permeability factors would explain this.

There are several reasons why enzymes or metabolic processes would exhibit different sensitivities to inhibitors in intact cells or extracts (see Chapter I-9). (1) The nature of the metabolism may be quite different in the two situations due to changes in the substrates used, altered concentrations of cofactors and coenzymes, different ionic environments, and many other factors. This is especially true when some very general process like respiration is involved. (2) Structural alterations and sometimes disruption of organized enzyme complexes may make the sensitive components more or less accessible to the inhibitor. (3) The binding of the inhibitor to nonenzyme material may be either increased or decreased in noncellular preparations, depending, for one thing, on the type of extract or degree of purification of an enzyme. Bergstermann and Mangler (1948) indeed pointed out that this might also explain their failure to find inhibition in a muscle brei. (4) Enzymes are often protected against the arsenicals by their substrates and within the cell the localized substrate concentration may be high, particularly in the case of enzymes operating in sequence. A number of other factors might be imagined. It is not necessary that extracts be more sensitive than cellular preparations. Ramachandran and Gottlieb (1963) found the glucose respiration of Caldariomyces cells to be inhibited
51% by 1 mM arsenite, whereas the respiration of an extract is inhibited only 7%.

Arsenite depresses the action potential of frog sciatic nerve after a lag period of 1-3 hr; it was thought this might be related to impenetrability of the surrounding membranes, but splitting the neural sheath has little effect on this (Schmitt et al., 1934). Furthermore, the action on the respiration is quite rapid, indicating adequate penetration. The rate of penetration may depend on the functional activity of a tissue, since arsenite is absorbed faster by stimulated brain in rabbits; if one sciatic nerve is stimulated there is more arsenite picked up by the contralateral side of the brain (Pigalev, 1940). The uptake of arsenite also increases with the fermentation activity in yeast (Diemair and Schülke, 1941). In experiments of this type one cannot be certain whether it is a matter of increased permeability or more binding sites.

The marine alga Valonia has often been used in permeability studies because it is possible to determine the concentrations of material in the protoplasm and the cell vacuolar fluid, so that surface binding can be eliminated. Brooks (1923, 1925) studied the entrance of arsenite into Valonia cells and found almost all taken up to be in the protoplasm, presumably because of binding to proteins. The uptake is minimal at an external pH of around 7. The effects of pH on penetration were discussed in terms of the ionization of arsenious acid, but there is obviously no simple relation between uptake and the concentration of the un-ionized form, and the author concluded that this is not the major factor in determining the rate of entrance. The uptake of arsenite was actually rather slow in these experiments and perhaps one might question whether permeability is measured under these circumstances. Most of the arsenite in the protoplasm must be bound and hence the uptake may be indicative only of the rate at which binding sites become available, particularly since 2 mM arsenite damages the cells. Although dead cells take up arsenite more rapidly than live ones, it is still a slow process. Ricks and Hoskins (1948) studied the effects of pH on the uptake of arsenite by flesh fly larvae and found that penetration decreases with rise in the pH (see accompanying tabulation). The less

<table>
<thead>
<tr>
<th>pH</th>
<th>Penetration rate (μg As/larva/hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>3.24</td>
</tr>
<tr>
<td>9.9</td>
<td>1.43</td>
</tr>
<tr>
<td>11.5</td>
<td>0.26</td>
</tr>
</tbody>
</table>

uptake at the high pH's could well be due to ionization of arsenious acid
in this case. The authors pointed out a factor which has been generally neglected: the effect of pH on the polymerization of arsenious acid. Although there is good evidence that polymerization occurs (page 602), there is no general agreement about the states of arsenious acid in the physiological pH range. Another factor of undoubted importance is the effect of pH on the structure and permeability of the surrounding membranes.

**Organic Arsenicals**

The rates of penetration of the substituted phenylarsenoxides into cells are dependent on the nature of the groups on the ring; indeed, in the case of the trypanosomes, this is the determining factor. Observations such as those on the accumulation of reduced tryparsamide by erythrocytes (Lourie *et al.*, 1935) are complicated by the binding of the arsenical to the membrane and stroma, but it is likely that penetration here is fairly rapid, half-uptake occurring in about 15 min, and that at least the initial uptake is not due to tight binding because the arsenical can be readily washed out. The penetration of phenylarsenoxide and *p*-aminophenylarsenoxide into yeast follows first-order kinetics for a time, but secondary processes disturb this simple relationship, the uptake becoming more and more irreversible (Janke and Garzuly-Janke, 1936). Conformation to first-order kinetics, of course, does not distinguish between adsorption, penetration, or chemical reaction. Similar results have been obtained with trypanosomes (Hawking, 1937, 1938) and the initial fixation of the arsenicals was claimed to occur within a few minutes. The very high cell/medium partition ratios (5000–10,000 for phenylarsenoxide and reduced tryparsamide) point to binding reactions as being more important than membrane penetration in determining the uptake kinetics. The effects of pH on the uptake of acidic phenylarsenoxides by trypanosomes have been studied in detail by Eagle (1945), but these results will be discussed in connection with the trypanocidal action (page 775); other investigations on pH effects, possibly relating to penetration, will be mentioned at that time. Essentially nothing is known about the rates of entrance of arsenicals into mammalian tissues, but the rapid penetration of nonionic lewisite through the skin has been demonstrated (Ferguson and Silver, 1947). The rate of movement through such tissues may be limited by reaction of the arsenical with the tissue proteins. It is probably safe to assume that the lipophilic alkyl and unsubstituted phenylarsenoxides pass rapidly through cell membranes, and that accumulation relates to the binding and reaction within the cells. The pentavalent phenylarsonates, being anionic within the physiological pH range, presumably penetrate less readily.
ACTIVE TRANSPORT

A few examples of the effects of arsenicals on transport processes are summarized in Table 6-9. In some cases the substance designated as transported actually may follow passively the substance truly moved, and in other cases truly active transport may not be involved (e.g., propionate, protamine, iodide, or water). The transport of glucose is difficult to investigate because of the Pasteur-like effect produced by the arsenicals and possibly the stimulation observed with rat intestine, and with other preparations not included in the table, is related to this. In any event, it is clear that active transports are quite sensitive to the arsenicals, and this must be of great importance in interpreting the effects of these substances on cellular metabolism, function, and growth. The problem as to whether the primary action is on the transport system or membrane permeability remains unsolved in most instances (see page 172 for a general discussion of this), but permeability effects are probably not so likely with the arsenicals as with some other SH reagents, like the mercurials, although Maizels (1951) interpreted the small effects on ion transport in erythrocytes as due to permeability changes inasmuch as some hemolysis is induced. Permeability changes may be caused not only by a reaction of inhibitors with the membrane components but, in the case of the arsenicals, by a depression of oxidative metabolism.

Is the inhibition of transport by the arsenicals due to some specific action on the transport system or merely to a general depression of energy generation from the cycle? This is a difficult question to answer. Some workers have attempted to obtain information on this by comparing the degrees of depression of transport and respiration, apparently on the assumption that a greater depression of the former would imply a selective action. Actually, in every instance in which such measurements have been made, the transport is inhibited more strongly (barley and mustard roots, frog skin, lymphosarcoma, and rabbit kidney slices), although seldom is the difference marked. Unfortunately, these results cannot establish either a relationship or the lack of one, since there is no necessity for a parallel inhibition of the transport and respiration. Only a fraction of the respiration may be involved in providing energy for transport, and in addition the rate of energy utilization for other activities will to some extent determine the effect of energy reduction on transports.* Butler (1953) noted that phosphate uptake in wheat roots can be depressed around 50% by 0.02 mM arsenite without altering protoplasmic streaming, indicating a selective effect on transport, a preferential requirement of transport for the energy available, or an action on the uptake unrelated to active transport.

* If transport were reduced without appreciable respiratory inhibition, some basis for attributing a selective effect on transport would be provided, but such has not been found experimentally.
### Table 6-9

**Effects of Arsenicals on Active Transport Processes**

<table>
<thead>
<tr>
<th>Transported Substance</th>
<th>Organism and tissue</th>
<th>Arsenical</th>
<th>Concentration (mM)</th>
<th>% Inhibition</th>
<th>$\Delta(X)_i$ (%)</th>
<th>General effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K^+$</td>
<td><em>Porphyra perforata</em></td>
<td>Arsenite</td>
<td>1</td>
<td>—</td>
<td>−60</td>
<td></td>
<td>Eppley (1958)</td>
</tr>
<tr>
<td>Barley roots</td>
<td>Arsenite</td>
<td>0.03</td>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td>Ordin and Jacobson (1955)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td>Maizels (1951)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2</td>
<td>75</td>
<td></td>
<td></td>
<td></td>
<td>Myers <em>et al.</em> (1963)</td>
</tr>
<tr>
<td>Human erythrocytes</td>
<td>Arsenite</td>
<td>2.8</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
<td>Mudge (1951)</td>
</tr>
<tr>
<td>Rat thymocytes</td>
<td>Arsenite</td>
<td>0.1</td>
<td>—</td>
<td>−21</td>
<td></td>
<td></td>
<td>Ulrich (1960)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>—</td>
<td>−60</td>
<td></td>
<td></td>
<td>Ulrich (1961)</td>
</tr>
<tr>
<td>Rabbit kidney slices</td>
<td>Arsenite</td>
<td>2</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td>Eppley (1958)</td>
</tr>
<tr>
<td>Rabbit heart mitochondria</td>
<td>Arsenite</td>
<td>1</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
<td>Levinsky and Sawyer (1953)</td>
</tr>
<tr>
<td>Rabbit kidney mitochondria</td>
<td>Arsenite</td>
<td>0.01</td>
<td>—</td>
<td>−5</td>
<td>T/M 17.4 to 17</td>
<td></td>
<td>Huf <em>et al.</em> (1957)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>—</td>
<td>−66</td>
<td>T/M 17.4 to 6</td>
<td></td>
<td>Maizels (1951)</td>
</tr>
<tr>
<td>$Na^+$</td>
<td><em>Porphyra perforata</em></td>
<td>Arsenite</td>
<td>1</td>
<td>—</td>
<td>+23</td>
<td></td>
<td>Eppley (1958)</td>
</tr>
<tr>
<td>Frog skin</td>
<td>Arsenite</td>
<td>1</td>
<td>94</td>
<td></td>
<td></td>
<td></td>
<td>Maizels (1951)</td>
</tr>
<tr>
<td>Frog skin</td>
<td>Arsenite</td>
<td>0.03</td>
<td>14</td>
<td></td>
<td>Na/K 2.09 to 1.97</td>
<td></td>
<td>Huf <em>et al.</em> (1957)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>61</td>
<td></td>
<td>Na/K 2.25 to 1.98</td>
<td></td>
<td>Maizels (1951)</td>
</tr>
<tr>
<td></td>
<td>Species</td>
<td>Transporter</td>
<td>Concentration (M)</td>
<td>Recovery (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>--------------------------</td>
<td>-------------</td>
<td>-------------------</td>
<td>--------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rb⁺</td>
<td>Mung bean roots</td>
<td>( \text{arsenite} )</td>
<td>0.0039, 0.0078, 0.012</td>
<td>25, 47, 62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H⁺</td>
<td>Mouse stomach</td>
<td>( \text{arsenite} )</td>
<td>0.34</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl⁻</td>
<td>Dog intestine</td>
<td>( \text{arsenite} )</td>
<td>0.5</td>
<td>100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Br⁻</td>
<td>Barley roots</td>
<td>( \text{arsenite} )</td>
<td>0.03, 0.1, 0.2</td>
<td>32, 75, 87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I⁻</td>
<td>Sheep thyroid slices</td>
<td>( \text{arsenite} )</td>
<td>1</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \text{HPO}_4⁻ )</td>
<td>Wheat roots</td>
<td>( \text{arsenite} )</td>
<td>0.02, 0.1</td>
<td>50, 100</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( 5 \text{ hr} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( 3 \text{ hr} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \text{Drosera tentacles} )</td>
<td>( \text{arsenite} )</td>
<td>0.01</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water</td>
<td>Potato slices</td>
<td>( \text{arsenite} )</td>
<td>0.01, 0.03</td>
<td>15, 60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propionate</td>
<td>Rat diaphragm</td>
<td>( \text{arsenite} )</td>
<td>5</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>Rat intestine</td>
<td>Oxophenarsine</td>
<td>0.01</td>
<td>St 2 ×</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References:
- Mudge (1951)
- Tanada (1956)
- Davenport and Jensen (1949)
- Ingraham and Visscher (1936)
- Ordin and Jacobson (1955)
- Slingerland (1955)
- Butler (1953)
- Arisz (1953)
- Hackett and Thimann (1950)
- Foulkes and Paine (1961)
- Manome and Kuriaki (1961)
<table>
<thead>
<tr>
<th>Transported substance</th>
<th>Organism and tissue</th>
<th>Arsenical</th>
<th>Concentration (mM)</th>
<th>% Inhibition</th>
<th>( \Delta(X)_i ) (%)</th>
<th>General effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td><em>Streptococcus faecium</em></td>
<td>Arsenite</td>
<td>10</td>
<td>6</td>
<td></td>
<td></td>
<td>Brock and Moo-Penn (1962)</td>
</tr>
<tr>
<td></td>
<td>Mustard roots</td>
<td>Arsenite</td>
<td>0.1</td>
<td>31</td>
<td></td>
<td></td>
<td>Wright (1962)</td>
</tr>
<tr>
<td></td>
<td>Rat diaphragm</td>
<td>Arsenite</td>
<td>10</td>
<td>—</td>
<td>T/M &lt; 10%</td>
<td></td>
<td>Christensen and Streicher (1949)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arsenite</td>
<td>25</td>
<td>—</td>
<td>T/M &lt; 56%</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gardner lymphosarcoma</td>
<td>Arsenite</td>
<td>5</td>
<td>97</td>
<td></td>
<td></td>
<td>Kit and Greenberg (1951)</td>
</tr>
<tr>
<td>Asparagine</td>
<td><em>Drosera tentacles</em></td>
<td>Arsenite</td>
<td>0.01</td>
<td>50</td>
<td></td>
<td></td>
<td>Arisz (1953)</td>
</tr>
<tr>
<td>Protamine</td>
<td>Leucocytes</td>
<td>Arsenite</td>
<td>1</td>
<td>40</td>
<td></td>
<td></td>
<td>Fischer (1950)</td>
</tr>
<tr>
<td>Phenol red</td>
<td>Flounder renal tubules</td>
<td>Arsenite</td>
<td>10</td>
<td>100</td>
<td></td>
<td></td>
<td>Forster and Taggart (1950)</td>
</tr>
<tr>
<td>( p )-AHA</td>
<td>Rabbit kidney slices</td>
<td>Arsenite</td>
<td>2</td>
<td>85</td>
<td></td>
<td></td>
<td>Cross and Taggart (1950)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td><em>Nitella clavata</em></td>
<td>Arsenite</td>
<td>10</td>
<td>0</td>
<td></td>
<td></td>
<td>Pramer (1956)</td>
</tr>
</tbody>
</table>

\( \Delta(X)_i \) is the change in the tissue concentration of the substance, \( \text{Na/K} \) is the ratio of the intracellular concentrations of \( \text{Na}^+ \) and \( \text{K}^+ \), T/M is the ratio of concentrations in the tissue and the medium. The per cent inhibition column refers to changes in the rates of transport.
Some information on the mechanism by which arsenite acts on $H^+$ transport may be found in the work of Davenport. Arsenite inhibits acid secretion, causes accumulation of pyruvate (Davenport and Jensen, 1949), but does not bring about detectable reduction of lipoate (Davenport et al., 1956). By the technique described (page I-504) and illustrated (Fig. I-10-6), it was found that arsenite markedly lowers the threshold for 2,4-dinitrophenol action (Davenport, 1955). The threshold is normally around 0.01 mM and arsenite lowers it to near 0.001 mM at a concentration which inhibits only 20%; this implies that arsenite inhibits the generation of ATP rather than interfering with its utilization, so the balance of evidence here points to the primary mechanism of transport inhibition being an inhibition of pyruvate oxidation.

Effects on the Kidneys

Studies on the toxicology of the arsenicals have shown that the kidney glomeruli and tubules may be to varying degrees directly damaged; renal function may be further disturbed by fluid and electrolyte imbalances. The first effect is perhaps a dilatation and swelling of the glomerular capillaries, followed by progressive tubular necrosis. The increased permeability of the glomerular capillaries leads to the proteinuria frequently observed. Some species, such as dogs and rabbits, may succumb to the renal lesions when organic arsenicals are administered (Gruhzit, 1935). Bunting and Longley (1940) made a thorough study of the renal changes brought about by tryparsamide in the rat. Rats were given increasing doses intravenously: 1 g/kg, which is half the tolerated dose, the first week; 1.5 g/kg during the second week; 2 g/kg during the third week; and 3 g/kg by the end of the month. The most prominent lesion 24 hr after the initial dose is a coagulative necrosis of the cells of the convoluted tubules with the appearance of pycnotic nuclei; if high doses are given initially, changes occur within 1 hr. The ascending limb shows the greatest damage. Glomerular injury is slight, but tryparsamide is known not to be a capillary depressant to the degree that the trivalent arsenicals are. At the end of the month there is no necrosis and the tubules are lined with mature epithelium, indicating the development of resistant cells (see page 765). The site of injury indicates the concentration of the arsenical during resorption to be the important factor, rather than a special sensitivity of these cells. Lewisite given intravenously produces glomerular congestion and cloudy swelling of the tubules within 1 hr, and tubular necrosis after 24 hr (Cameron et al., 1946). It is somewhat surprising that the urine volume decreases in arsenical poisoning (Von Boeck, 1871; Araki, 1893; Jastrowitz, 1908) since other SH reagents, such as the mercurials, usually produce diuresis, and there appears to be no particular inhibition of ion and water resorption by the arsenicals. Much of the oliguria might be due to obstructive swelling
of the glomerular capillaries and tubules, but the reduction of the blood volume and blood pressure may also contribute. Renal transports in isolated preparations have been shown to be depressed (Table 6-9), but it should be noted that in most of this work, especially with regard to the ions, transtubular transport is not determined but presumably only the intracellular exclusion of Na\(^+\) and accumulation of K\(^+\). The immediate effects from subtoxic doses of the arsenicals on these important renal transports in vivo have never been investigated.

**TISSUE FUNCTIONS**

The effects of the arsenicals on certain tissues will be discussed in this section, not only because of the importance of attempting to correlate functional and metabolic changes, but in order to provide a basis for understanding some of the toxic effects in arsenical poisoning.

**Nervous System**

It is surprising, in view of the metabolic actions of the arsenicals, that central nervous system effects are generally minimal and have often not even been mentioned in toxicological studies, since the nerve cells depend on pyruvate oxidation and the cycle for their energy supply. A characteristic result of chronic poisoning is peripheral neuritis, but central function is little disturbed. In acute poisoning there may be respiratory depression, which could arise from an action on the central respiratory pathways, and terminal convulsions, but these latter are more probably anoxic in origin. The specific effects of the pentavalent arsenicals on the optic nerve will be discussed later (page 716). Most workers would probably argue that the lack of central effect is due to poor penetration of the arsenicals into the brain or the nerve cells, and certainly the asenicals occur in the brain in lower concentration than in most other tissues (page 780). Morishima (1900 a) observed that arsenite given by intracerebral injection to rabbits is much more toxic than by other routes, and that the animals exhibit marked paralysis and no diarrhea. Koppanyi and Sperling (1947) found that neither arsenite nor dimercaprol produces central effects at certain dosage levels, but when rabbits receive dimercaprol intramuscularly and arsenite 10 min later intravenously there is rapid development of motor excitation and parasympathetic stimulation. This might be due to the greater lipid solubility of the arsenite-dimercaprol complex and consequently its rapid penetration into the central nervous system. However, in the light of certain enzyme responses to mixtures of arsenite and dimercaprol (page 645) and the fact that injection of the complex into rabbits does not produce these effects, it might be well to be cautious in interpreting these results.
Sklarek (1866) noted that arsenite abolishes sensation and reflex activity in frogs, and later reduces voluntary motor activity. Ringer and Murrell (1878) reinvestigated this and found the loss of voluntary power to occur well before the loss of reflexes. The muscles in a frog paralyzed by arsenite still contract to direct electrical stimulation; furthermore, the motor nerves conduct normally during paralysis (Lesser, 1878 c). Thus the block must be either central or on the neuromuscular junction; Ringer and Murrell assumed the former. The data necessary to locate the site of action have not been reported. Arsenite seems to have little effect on nerve axons. Makarov (1927) found complete conduction blockade only after 30-70 hr in arsenite concentrations as high as 75-300 mM, but the results of Lendle and Reinhardt (1931) are quantitatively quite different (see accompanying tabulation). The nerves in this work are reasonably sensitive

<table>
<thead>
<tr>
<th>Exposure time (hr)</th>
<th>Concentration for conduction block (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nerve</td>
</tr>
<tr>
<td>8</td>
<td>15.4</td>
</tr>
<tr>
<td>24</td>
<td>0.51</td>
</tr>
<tr>
<td>48</td>
<td>0.11</td>
</tr>
</tbody>
</table>

but the time required to act is very long. This could be explained on the basis of slow penetration, but it is seen that muscle behaves similarly. Schmitt et al. (1934) showed that whereas arsenite depresses nerve respiration rapidly, the effects on the action potential are delayed (see accompanying tabulation). That the neural sheath presents a barrier to the arsenite

<table>
<thead>
<tr>
<th>Arsenite (mM)</th>
<th>Extinction time for action potential (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>&gt;24</td>
</tr>
<tr>
<td>0.1</td>
<td>10</td>
</tr>
<tr>
<td>0.2</td>
<td>6.8</td>
</tr>
<tr>
<td>1</td>
<td>4.7</td>
</tr>
<tr>
<td>2.5</td>
<td>3.7</td>
</tr>
<tr>
<td>11</td>
<td>3.5</td>
</tr>
<tr>
<td>100</td>
<td>2.6</td>
</tr>
</tbody>
</table>

was eliminated by showing that a lag period exists after splitting of the sheath. There are probably two reasons for the lag period. One is the fact
that relatively little ATP is required for the ion pumps in relatively inactive nerve, and respiration must be depressed to a fairly low level before it would fail to provide sufficient ATP. The second is that the ion distributions involved in the membrane potentials are ultimately but not immediately dependent on oxidatiye metabolism, failure in conduction occurring only when sufficient K\(^+\) has been lost from the cells, this requiring a long time because very small amounts of K\(^+\) leave the cell during each impulse. Jenerick (1957) similarly found a very slow action of 5 m\(M\) arsenite on sciatic nerve, conduction block occurring when the resting potential has fallen 30–40\%, excitability being reduced after the action potentials are lowered 10–20\%. The intraaxonal injection of \(5.6 \times 10^{-8}\) ml 27 m\(M\) arsenite into giant axons of the squid (this corresponds to \(1.5 \times 10^{-6}\) millimole/mm) has no effect on the action potential (Brady et al., 1958). This can be calculated to give an internal concentration of about 3 m\(M\), which should be quite adequate to block oxidative metabolism.

**Skeletal Muscle**

We have seen that paralysis in frogs given arsenite is not primarily muscular (Ringer and Murrell, 1878). However, it was noted that rigor mortis occurs earlier in poisoned animals, so the authors concluded that the muscles are affected. Further evidence that muscle can be altered in vivo was obtained in an experiment in which muscle excitability was lost within 13 hr in a poisoned limb but only after 34 hr in a ligatured limb. Heffter (1893) reported a moderate rise in muscle lactate after death from arsenite but did not observe rigor, while Nonnenbruch et al. (1936) found decreases in muscle hexose-P, ADP, and phosphorylative ability in arsenite-poisoned rabbits. Stimulated isolated frog muscle in 40 m\(M\) arsenite shows a steady increase in tone until full contracture develops (termed a Lundsgaard effect) and the muscle is inexcitable* (Gernay and Lecomte, 1948). It was assumed that the block of pyruvate oxidation was responsible for the contracture, but no evidence was presented. Neoarsphenamine causes contracture of frog muscle and thiamine prevents this (Pick and Holland, 1951), but it is doubtful if this antagonism is in any way related to the keto acid oxidases. The contracture produced by oxophenarsine is of the slowly developing type (Kuschinsky and Lüllmann, 1954), and in the rat diaphragm there is no change in the magnitudes of the resting or action potentials while the muscle passes into contracture, although depolarization occurs when contracture is complete (Muscholl, 1958). Thus contracture appears to be a common effect of the arslenicals, occurring

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* The potassium salt of arsenite was used here for some obscure reason and, since this ion can induce contracture, one does not know how much of the effect was due to the arsenite. However, oxophenarsine at 12.6 m\(M\) produced a similar effect.
in cardiac muscle also (page 710), and is not the result of membrane depolarization. However, soaking frog muscle in 10 mM arsenite abolishes the resting potential after 4 hr, and it was postulated that pyruvate oxidation and the operation of the cycle are involved in the maintenance of the potential (Ling and Gerard, 1949). Arsenite at concentrations as high as 100 mM does not alter muscle after-potentials (Macfarlane and Meares, 1958). Relatively little work has been done on glycerinated muscles and preparations of the contractile proteins, but it has been observed that oxophenarsine at 0.1 mM reversibly abolishes the ATP-induced contraction of nonconducting rabbit psoas muscle (Korey, 1950), at 0.5 mM inhibits the contraction of actomyosin and the polymerization of actin (Turba and Kuschinsky, 1952), at 0.8 mM inhibits the ATPase activity and the superprecipitation of actomyosin (Mugikura et al., 1956), and at 0.7 mM inhibits myosin ATPase and the formation of actomyosin in parallel fashion (Bárány and Bárány, 1959a). The viscosity and Ca++-binding of actin are not much altered by 2 mM oxophenarsine, in contrast to the potent effects exerted by the mercurials (Bárány et al., 1962). It is difficult to extend these results to intact muscle but clearly the arsenicals can directly affect certain contractile processes.

**Smooth Muscle**

Despite the well-known effects of arsenicals on the capillaries and the gastrointestinal tract, no detailed studies of the actions on these smooth muscles, or others, have come to my attention. The intestinal edema, the serous and mucosal hemorrhages, and the gastric and intestinal erosions seen in arsenic poisoning (Cameron et al., 1946) have been generally assumed to be caused by vascular damage rather than a major direct effect on the gastrointestinal tract. The contractile amplitude of isolated rabbit intestine is depressed by 0.1 mM arsenite, while 4 mM abolishes contractions with the intestine in a relaxed state (Joachimoglu, 1915). Alanis (1948) pointed out that oxophenarsine produces the same effects on smooth muscle as on skeletal muscle and the heart, and showed that the isolated rabbit intestine and the uteri of various species respond by an initial contraction followed by relaxation and loss of rhythmic activity. It may be recalled that, in the early work of Lesser (1878c) and Pistorius (1883) on the *in vivo* responses of the intestine to arsenite, an initial increase in peristalsis or tetanic contractions, or both, was observed, these effects not being much altered by denervation. One might imagine the initial stimulation to be due to histamine release, since the mercurials have been shown to do this, but arsenite does not release histamine from the cat intestine (Bachmann, 1938) and, in fact, inhibits the release of histamine by the releaser, Compound 48/80 (VanArsdel and Bray, 1961), and anaphylaxis (Chakravarty, 1962). The
effects of the arsenicals on blood vessels will be discussed in a later section (page 726).

Heart

The depressant action of arsenite on the hearts of frogs and mammals was first observed by Sklarek (1866) and Cunze (1866), who showed that although the heart is depressed in vivo, the atria are less sensitive than the ventricles and may continue to beat for several hours after death. Ringer and Murrell (1878) confirmed that the heart continues to beat after death and concluded that it is more resistant than the central nervous system to arsenite. Unterberger and Boehm (1874) then demonstrated that the injection of arsenite into dogs and cats slows the heart directly and not by an effect mediated through the cardiac nerves, and that it does not significantly alter the cardiac response to vagal stimulation. Lesser (1878 a) reported a progressive slowing of hearts in arsenite-poisoned frogs, the hearts stopping in diastole; however, in several minutes the ventricles may begin to beat with a rhythm of their own. The injection of arsenite in small doses into rabbits and dogs may produce an initial elevation of the cardiac rate, while the contractile amplitude is only depressed, the depression occurring also when the vagi are cut (Lesser, 1878 b).

There is a disconcerting variation in the reported responses of the isolated frog heart to arsenite. Loewi (1897) found the heart to be depressed by concentrations as low as 0.0077 mM, and to be stopped in 100 min by 0.039 mM, in 50 min by 0.15 mM, and in 30 min by 1.5 mM. In contrast, Sivertsev (1938) claimed that 0.1 mM arsenite does not affect either rate or output, while 0.3–0.7 mM reduces the output by 65%. In some instances the rate is readily depressed (Holzbach, 1912), but in most work the contractile amplitude is reduced with little or no change in the rate, at least until terminal failure (Lesser, 1878 a; Loewi, 1897; Joachimoglu, 1915; Sivertsev, 1938). There is general agreement that the heart stops in diastole, even with high concentrations of arsenite (Vogt, 1930), but it may be put into contracture by electrical stimulation (Méndez, 1946). Almost all workers have found the effects to be irreversible. An initial and temporary stimulation of the heart by arsenite has been reported in isolated preparations from frogs (Zondek, 1920; Vogt, 1930), silkworm (Campbell, 1926), and rat (Webb and Hollander, 1959). This is rather characteristic of SH reagents but the mechanism is unknown.

The stimulating effect of epinephrine on the rate of the frog heart, both in vivo and isolated, was claimed to be blocked by arsenite (Holzbach, 1912). The injection of epinephrine into poisoned frogs temporarily raises the blood pressure but does not change the cardiac rate, while isolated hearts depressed by arsenite have their output restored by epinephrine even though the rate remains low. On the other hand, Vogt (1930) reported that 0.77 mM arsenite does not interfere with the stimulatory action of
epinephrine, although 3 mM weakens it, and Nickerson and Nomaguchi (1950) also found 0.5 mM arsenite to be without effect on the chronotropic response to epinephrine. Actually, the situation is probably this: the initial stimulation by epinephrine may not be appreciably reduced by arsenite (assuming that the heart is not too much depressed), but the stimulation is temporary and actually promotes more rapid failure, as is the case with a variety of inhibitors as well as anoxia. Frog hearts depressed by arsenite are also stimulated by Ca++ but further depressed by K+ (Zondek, 1920).

Some slight progress has been made toward an understanding of the possible mechanisms involved by investigating the changes in the electrical activity of the heart. Electrocardiographic disturbances, including prolongation of the qrs interval, lowering of the st segment, and ectopic activity, have been noted in human arsenical poisoning (Zettel, 1943), and in rabbits poisoned with arsenite one finds electrocardiographic alterations corresponding to cardiac damage (Petrucci and Boggio-Gilot, 1955). It is, of course, difficult to interpret such changes in terms of direct actions on the heart, since many secondary effects may be involved, but it seems likely that at least some of the electrocardiographic effects are direct. Goldenberg and Rothberger (1937) studied the electrical activity of dog hearts depressed by the perfusion with 1–2.5 mM arsenite, and claimed that there is a tendency for aberrant after-potentials to occur following relatively normal action potentials. It is clear from their records, however, that the true action potential is shortened and reduced in amplitude, although the period of electrical variation may be prolonged. Recording intracellularly with microelectrodes in the rat atrium, it has been found that the effects on membrane potentials are not marked when the contractile activity is quite severely depressed (see accompanying tabulation) (Webb and Hol-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>% Change from 1 mM arsenite (at 21–40 min)</th>
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<tbody>
<tr>
<td>Resting potential</td>
<td>- 1.8</td>
</tr>
<tr>
<td>Action potential</td>
<td>- 8.5</td>
</tr>
<tr>
<td>Action potential duration</td>
<td>- 13.0</td>
</tr>
<tr>
<td>Action potential area</td>
<td>- 18.7</td>
</tr>
<tr>
<td>Repolarization rate</td>
<td>+ 1.6</td>
</tr>
<tr>
<td>Developed tension</td>
<td>- 64.1</td>
</tr>
<tr>
<td>Conduction rate</td>
<td>- 2.4</td>
</tr>
</tbody>
</table>

lander, 1959). Of all the metabolic inhibitors examined, arsenite produced the most specific contractile depression; that is, since little of the contractile change can be attributed to alteration in the characteristics of the membrane
potentials, one must assume that arsenite acts either directly on the contractile systems or on some aspect of excitation-contraction coupling.* There is invariably some stimulation of the contractions at 2–5 min. The atria usually stop in diastole, but some begin to shorten before this; all eventually go into contracture, but more slowly than with iodoacetate, and the ultimate tension reached is probably not as great. The effects observed on glycerinated and actomyosin preparations of skeletal muscle, if they can be extended to the heart, could explain some or all of the cardiac contractile changes. And yet arsenite has been shown to cause accumulation of keto acids and to deplete ATP in the heart; these actions must play a role in the contractile depression, but apparently not through membrane changes.

Two questions come up in this work and are difficult to answer: why does it require a relatively high arsenite concentration to depress mammalian hearts, and why does it take so long for the depression to be manifest? If arsenite penetrates readily into the heart cells, a concentration of 1–2 mM should almost completely block pyruvate oxidation, the operation of the cycle, and a number of other metabolic pathways, if one can use the data obtained on isolated enzyme systems. The heart is generally considered to be a tissue energetically dependent on the cycle. The heart can utilize fatty acids, but arsenite at this concentration should not only prevent the entry of acetyl-CoA into the cycle (by blocking at the α-ketoglutarate step) but also inhibit oxidation via the helix. The delay in effect cannot be adequately explained on the basis of the time required to deplete ATP stores, since low uncoupling concentrations of 2,4-dinitrophenol (around 0.01 mM) produce contractile failure in a much shorter time than arsenite. Is it possible that the lipoate or other vicinal SH groups in the intact cell may be relatively inaccessible to arsenite, as in some electron transport systems (page 661)? Thus higher concentrations of arsenite and longer periods of action would be required for depression, compared to systems isolated from the cell.

**EFFECTS OBSERVED IN THE WHOLE ANIMAL**

We shall be concerned in this section primarily with the toxic effects of the arsenicals in animals and with the dependence of these effects on the metabolic disturbances produced in the tissues. The toxicology of arsenite and the simpler arslenoxides will be emphasized inasmuch as it is with the metabolic actions of these that we are best acquainted. The organic penta-

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* One can also assume that ATP is compartmentalized and the ATP used in membrane events is from a different source than that used in contraction, pyruvate oxidation being essential for the generation of ATP in the latter case, but actually what little evidence is available is against this.
valent arslenals undoubtedly exert toxic effects after being reduced to the corresponding arsenoxides, but at least in some cases appear to produce unique and characteristic damage to certain tissues. It will not be necessary to treat arsenical poisoning in detail because adequate accounts are available in the toxicology texts and in Buchanan’s “Toxicity of Arsenic Compounds” (1962).

**Toxicology of Arsenite**

*Acute poisoning* by arsenite presents somewhat different patterns in various species, but the most characteristic picture includes the following: (1) vasodilatation and hyperemia, especially splanchnic, leading to a fall in the blood pressure and possibly shock (Dogiel, 1881; Kionka, 1911; Holzbach, 1912; Tinno et al., 1948; Hanna and McHugo, 1960); (2) escape of plasma from the capillaries leading to edema (see page 727) (Magnus, 1899); (3) generalized gastrointestinal distress with pain, vomiting, and diarrhea (Araki, 1893; Pistorius, 1883), so that it is sometimes difficult to distinguish arsenic poisoning and duodenal ulcer without roentgenography (Adelson et al., 1961); (4) reduction of urine volume to final anuria, the urine being albuminous and bloody, with evidence of glomerular and tubular damage (see page 705); (5) depression and eventual paralysis of respiration, this often being the cause of death (Lesser, 1878 c); (6) loss of voluntary movement and paralysis of central origin (Ringer and Murrell, 1878); and (7) hypothermia, which is often marked, the body temperature falling 5–10° (Norris and Elliott, 1945). A number of other changes, referable mainly to disturbances in the autonomic nervous system and the circulation, also occur. The cardiac effects have been discussed in detail (page 710). In the terminal stages, reflex activity is very depressed or absent, the blood pressure is at shock levels, and the respiration is markedly reduced; convulsions may occur but are probably anoxic in origin.

*Chronic poisoning* by arsenite may exhibit the above effects in modified form and, in addition, the following have been observed: (1) anhydremia due to excessive losses of fluid from the blood into the tissues and the gastrointestinal tract, associated with electrolyte imbalance; (2) inflammation of the eyes and respiratory tract, as in acute coryza; (3) dermatoses of various types, including hyperpigmentation, hyperkeratosis, desquamation, and loss of hair; (4) hematopoietic depression with mitotically abnormal erythroblasts, fall in leucocyte count, and occasionally aplastic anemia; (5) anorexia and loss of weight; (6) varying degrees of liver damage with jaundice, portal cirrhosis, and ascites; and (7) sensory disturbances and peripheral neuritis. It is very difficult, especially in chronic poisoning, to separate the direct actions of arsenite from a variety of secondary effects, but there is no doubt that the over-all pattern of poisoning represents the complex result of many interrelating processes.
Toxicology of the Organic Arsenoxides

Most of our knowledge comes from the extensive reports on the toxic reactions to the clinically used arsenicals, and since complete descriptions of these reactions are given in pharmacology texts, only a brief summary will be given here. It may be noted that generally the effects of the arsenoxides are similar, if not identical, to those of arsenite, so that essentially all the reactions given in the previous section may be observed. None of these, of course, occurs frequently at the therapeutic dosage levels; indeed, some of the reactions occur so infrequently that they may well be sensitivity responses of an allergic type. The differences between the effects of the arsenoxides and arsenite are more interesting. It appears, for example, that the arsenoxides exert relatively less action on the circulation and the gastrointestinal tract; animals may die from oxophenarsine poisoning without obvious vasodilatation or signs of intestinal damage. It is a matter of degree only, however, since patients often exhibit diarrhea after intravenous oxophenarsine, and lewisite given systemically is capable of causing severe mucosal hemorrhage with gastric and intestinal erosions (Cameron et al., 1946). Some species are particularly sensitive to the central effects while others more often die of renal lesions (Gruhzit, 1935). In dogs and man the cerebral blood vessels seem to be more affected than the vessels elsewhere by oxophenarsine, and considerable edema and hemorrhage may be present in the brain with little evidence of vascular derangement elsewhere (Sexton and Gowdey, 1947). Lewisite is definitely more toxic than arsenite to the liver and kidney, is more apt to cause pulmonary lesions (e.g., edema and alveolitis), and has relatively less effect on the heart or central nervous system (Cameron et al., 1946). Some of the differences between arsenite and the arsenoxides may be due to different distributions in the tissues, but at the present time one cannot be certain that they produce identical metabolic lesions. It should not be thought that all the phenylarsenoxides produce the same toxic effects. Hurst (1959) has shown that Melarsen oxide in single injections to monkeys causes a hemorrhagic necrosis at specific sites in the central nervous system, particularly the external geniculate bodies and the ventral pons, while phenylarsenoxide does not produce such lesions, perhaps because it kills through hepatic and renal damage. Nor does arsenite cause these lesions. Possibly the effects of Melarsen oxide are of vascular origin.

A few brief remarks relative to the effects of the arsenicals on the liver will not be out of place here because, although the mechanisms are not understood, a metabolic basis is likely which might repay further investigation. Vogel (1928) reported that arsenic is often found in abnormal amounts in patients with jaundice, stated that it might be an etiological factor in cirrhosis and hepatic atrophy, perhaps being responsible for the sporadic outbreaks of liver disease, and felt that the liver bore the brunt of arsenic
toxicity. Rössing (1941) found a picture in acute arsenic poisoning of dam-
age to the reticuloendothelial system, necrosis of the hepatic cells, and
various degrees of acute yellow atrophy; in chronic poisoning there is fatty
infiltration simultaneous with progressive necrosis and congestion of the
bile ducts. Portal cirrhosis and ascites have been attributed to the prolonged
use of Fowler’s solution (Franklin et al., 1950), but the etiological responsi-
ability of arsenic here is uncertain. Intensive intravenous arsenotherapy
in patients has been reported to cause damage to the liver, and certainly
large doses of arsphenamine in rabbits can induce a marked necrosis with
fatty degeneration, but the question has arisen as to whether the syphilitic
infection is the cause. Indeed, Thomas and Olansky (1946) stated that
in intensive 5-day arsenotherapy no evidence of damage to the liver could
be obtained and, in fact, some improvement was often noted, indicating
the presence of syphilitic hepatitis. Lewisite causes striking changes in the
liver within a few hours in most species, the sequence of events being briefly:
edema and congestion, distention of bile ducts, necrosis of gall bladder
mucosa, desquamation of epithelium, mitochondrial disintegration — by
4–6 hr there is cloudy swelling and biliary hemorrhages and by 12 hr the
liver is mottled and necrotic, the cells vacuolated, and the nuclei pycnotic
(Cameron et al., 1946). It was thought by Foulerton (1921) that certain
arsenicals have a special affinity for lipids, are carried to the liver in the
circulating fat, and disrupt lipid metabolism in the liver, but it seems
unlikely that a unique mechanism must be postulated. Whatever the ultimate
origin of the hepatic damage, these effects on the liver must contribute
to the observed changes in blood composition.

Toxicology of the Organic Arsonic Acids

The relatively low toxicity of the pentavalent arsenicals, such as atoxyl,
tryparsamide, and carbarsone, indicates that these compounds are not
immediately and quantitatively reduced to their corresponding arsenoxides,
and casts some doubt on the generally accepted theory that these pent-
valent arsenicals produce all their effects following reduction. It is known
that a fairly large fraction of the administered pentavalent arsenical is
fairly rapidly excreted as such. It is probable that the penetration and
distribution of the tri- and pentavalent arsenicals in the tissues are quite
different, which complicates an interpretation of their actions based solely
on metabolic inhibition. Arsonic acids very seldom cause the typical reac-
tions seen with the trivalent arsenicals, even during chronic administration;
e.g., with tryparsamide and carbarsone, the occurrence of liver damage,
renal disturbances, dermatitis, or diarrhea is rare, and usually no indication
of definite vascular effects is found. This is all the more surprising when
one considers the relative toxic doses of the tri- and pentavalent arsenicals.
For example, the ratio of the maximal tolerated doses of tryparsamide and
reduced tryparsamide in mice is 30 by subcutaneous and intraperitoneal injection, and 120 by intravenous injection (Fulton and Yorke, 1943). One might expect that a small fraction of the tryparsamide reduced in the body would produce some typical trivalent toxicity. Tryparsamide causes relatively little liver damage even when administered over long periods of time to human subjects. In 34 of 66 patients studied and receiving on an average 532 g tryparsamide in 193 injections over 7.4 years, only slight impairment of liver function was observed, and when jaundice occurred it disappeared rapidly (Kopp and Solomon, 1943). The toxic dose of carbarsone oxide is much less than for carbarsone, and furthermore the oxide systemically causes acute liver damage and hemorrhagic pulmonary edema, reactions not seen with carbarsone (Anderson et al., 1947).

The pentavalent arsenicals occasionally induce optic damage and blindness, this being the most serious reaction to tryparsamide and atoxyl, although it does not occur with carbarsone, showing that it is not the pentavalent nature alone which is responsible; no trivalent arsenicals produce this. It has been known since 1905 that atoxyl causes optic damage and a similar action of tryparsamide was discovered in 1915. Young and Loevenhart (1924) believed that a p-amino group is necessary for this effect, since neither o- nor m-amino compounds are active, but Longley et al. (1942) showed that some arsenicals without an amino group, or with a m-acetyl-amino group, can bring about optic damage. We do not know the structural requirements, nor do we understand the specificity of these substances for the optic system. The nature of the damage is as follows: progressive concentric contraction of the visual field; cloudiness of vision and an occasional shimmering effect, but with no scotomatas and no disturbance of reflexes; pallor of the optic disk and some narrowing of the retinal vessels; acute degeneration of the retinal ganglion cells, leading to a marked decrease in number, and of the innermost portion of the nuclear layer of the retina, with some damage to rods but none to cones; and proliferation of glial cells in the retina. The peripheral portions of the optic nerve are the most sensitive, but the optic nerve itself may exhibit degeneration and demyelinization; there is little evidence of central damage. Almost complete optic atrophy may be induced in the most severe cases. This is not at all the same optic injury produced by iodoacetate (Sorsby et al., 1957). It appears that this selective action on the optic system is due to either (1) a peculiar permeability of the cells involved to these pentavalent arsenicals, the intracellularly active form being trivalent, or (2) a direct action of the pentavalent forms, unassociated with inactivation of SH enzymes. Most enzymes which have been investigated, with the possible exception of certain lipases, are quite resistant to atoxyl and tryparsamide, so that the mechanism, if direct, must for the present remain obscure.
Toxic and Lethal Doses

Selected data on toxicity are presented in Table 6-10 and provide some comparative assessment of the relative potencies. Although there is a good deal of variability, the average ratio of toxic potencies of the trivalent and pentavalent arsenicals is 58 with regard to intraperitoneal administration to mice (Eagle and Doak, 1951). The variation is mainly among the pentavalents. The trivalent arsenicals are roughly of equivalent toxicities, but possibly the additional factor of reduction increases the variability of pentavalent activity. Such toxic doses should not be construed as accurate and invariable because many factors may alter the sensitivity of animals.

The time factor is very important in poisoning by the arsenicals. Thus the LD$_{50}$ determined at 2 hr may be greatly different from that at 24 hr. In rabbits, arsenite given subcutaneously kills within 10–12 hr at 6.6 mg/kg, within 1 hr at 26 mg/kg, and within 30 min at 66 mg/kg (Morishima, 1900 a); in frogs, 20 mg/kg kills in 2 days, whereas 300–600 mg/kg kills in around 1 hr (Ringer and Murrell, 1878). The susceptibility of mice to arsenite varies with age and sex, and sexual intercourse has been shown to increase resistance to arsenite, around 50% greater dose being required in males and 25% greater in females, either the cause or the effects falling off with age (Agduhr, 1937). Hormonal levels may also be of importance, hyperthyroidism increasing the susceptibility of mice to phenylarsenoxide and neoarsphenamine (Dybing, 1948), and ACTH or adrenal extracts decreasing susceptibility some 35–40% (Beck, 1951). Tumor-bearing animals are somewhat more resistant than normals to oxophenarsine; at a certain dose the survival was increased from 26% to 83% (Beck and Gillespie, 1954). This effect is not due to accumulation of the arsenical in the tumor tissue since analyses showed no specific uptake. In connection with the variability of toxic doses, perhaps it is well to remember that As$_2$O$_3$ often contains very significant amounts of Sb$_2$O$_3$, which can contribute to the effects produced, particularly the vascular and gastrointestinal (Harrisson et al., 1958).

The relation of arsenical structure to toxic potency presents many intriguing problems in interpretation; much of the work has been well summarized in the review article of Eagle and Doak (1951). The unsubstituted phenylarsenoxide is highly toxic to all types of cells studied and shows relatively little selective effect on infective parasites. Many phenylarsenoxides have been examined in an effort to find compounds more selectively parasiticidal (Table 6-11). Ring substituents almost invariably reduce the toxicity. An unfortunate fact preventing satisfactory explanation of variations in activity with structure is the almost complete lack of data on the effects of structural changes on the reactivity of the arsenicals with enzyme systems. In other words, when groups are introduced into the phenylarsenoxide ring, the toxic or parasiticidal activity may be altered due either
<table>
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<tr>
<th>Arsenical</th>
<th>Animal</th>
<th>Route&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Parameter&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Dose (mg/kg)</th>
<th>Reference</th>
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<tr>
<td>Arsenite</td>
<td>Mouse</td>
<td>SC</td>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>10.8</td>
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<td>Parameter</td>
<td>Dose (mg/kg)</td>
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<td>Yorke et al. (1931 a)</td>
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<td>34</td>
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<td>50</td>
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$^a$ Routes of administration: IV = intravenous, SC = subcutaneous, IC = intracutaneous, IM = intramuscular, IP = intraperitoneal.

$^b$ Parameters measured: MLD = minimal lethal dose, TD = tolerated dose, LD = lethal dose (degree unspecified), $L_D_x$ = dose to kill $x\%$. 
to a change in the reactivity with the cellular components, or to a change in the ability to penetrate into the cells. We have no adequate evidence relative to the former, but there is positive evidence that penetrability is a major factor. Let us first consider what little is known about the influence of structure on the reactivity with enzymes. Barron and Singer (1945) found that phenylarsenoxide and several derivatives comparably inhibit succinate oxidase (see accompanying tabulation). Certainly one can detect

<table>
<thead>
<tr>
<th>Phenylarsenoxide</th>
<th>% Inhibition of succinate oxidase (at 0.042 mM)</th>
<th>Relative activity toward:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Mice</td>
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<td>100</td>
</tr>
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<td>p-COOH</td>
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<td>41</td>
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<tr>
<td>p-NH₂</td>
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<tr>
<td>p-COMH₂</td>
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<td>10</td>
</tr>
<tr>
<td>3-NH₂-4-OH</td>
<td>51</td>
<td>7</td>
</tr>
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</table>

no correlations here and the wide variations in activity cannot be readily explained by these data. However, succinate oxidase can be considered as a model system only, since other enzymes in the cells are more sensitive and important. The authors also found that phenylarsenoxide and the p-COOH derivative are equally inhibitory to malate dehydrogenase. Rothschild et al. (1954) reported that choline oxidase is inhibited to the same degree by the phenylarsenoxides in the table above, but such a high concentration (1 mM) was used that accurate comparisons cannot be made. The p-COOH and p-NH₂ derivative exert identical inhibitions on amine oxidase (Singer and Barron, 1945). Other data on less important enzymes are equally unrewarding. There seems to be no evidence for marked differences in enzyme inhibitory action, but ideally studies should be made on the keto acid oxidases.

Turning to the explanation of variations in toxicity based on permeability, we are on more solid ground. First, we shall see that penetration of an arsensical into protozoans is a very important factor in determining the susceptibility. Second, Hogan and Eagle (1944) showed that the amount of arsensical taken up by erythrocytes is proportional to the toxicity of the arsensical (Fig. 6-6). A similar relationship was demonstrated for liver and kidney following injection of equivalent doses of various arsenoxides, the more toxic the compound the greater the amount found in the tissue (see accompanying tabulation). Finally, the amount of arsensical taken up by trypanosomes is also proportional to the trypanocidal activity (Fig. 6-8) (Eagle and Magnuson, 1944). Although these results might be interpreted as due to different degrees of binding, in view of the lack of evidence for
### Table

<table>
<thead>
<tr>
<th>Phenylarsenoxide</th>
<th>Relative toxicity</th>
<th>Tissue As (µg/100 g) in:</th>
<th>% Excreted</th>
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<td></td>
<td></td>
<td>Liver</td>
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</tr>
<tr>
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<td>100</td>
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<td>86</td>
<td>109</td>
<td>53</td>
</tr>
<tr>
<td>( p-NH_2 )</td>
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</tr>
<tr>
<td>( 3-NHCONH-4 )</td>
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<tr>
<td>( p-NHCOCH_3 )</td>
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<td>17</td>
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<td>( p-CH_3NH_2 )</td>
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<tr>
<td>( 3-NH_2-4-OH )</td>
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<td>2</td>
<td>9</td>
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</tbody>
</table>

variable binding it is more likely that penetration into the cells is the major factor. It has also been shown that the activity and the amount bound of various arsenicals, especially those with acidic substituents, vary with the pH in parallel fashion (see page 777). We must then conclude that when substituent groups are placed on the phenylarsenoxide ring, the ability

![Graph](image-url)

**Fig. 6-6.** Binding of arsenicals to erythrocytes as related to their toxicity. The incubation time was 10 min. All the arsenicals are substituted phenylarsenoxides. (From Hogan and Eagle, 1944.)
of the substance to penetrate into cells is modified and consequently the toxic potency is changed. Of course, other factors must also contribute — e.g., the rate of metabolism or inactivation of the arsenicals, the rate of excretion, the binding to metabolically inert material — but these must play a minor role. The fact that substitution of phenylarsenoxide generally reduces the toxicity might be expected on the basis that bulky or charged groups would reduce penetration. Further discussion of structure-activity relationships will be found in the section on the parasiticidal properties of the arsenicals (page 739).

**Effects of Subtoxic Doses**

No account of the actions of the arsenicals would be complete without at least brief mention of the responses attributed to the administration of low doses. Arsenic has been used in veterinary medicine since at least the fifteenth century, not only in the treatment of various diseases, but also to make the animals strong and healthy. During the last century numerous reports appeared claiming that the chronic feeding of small doses of various preparations of arsenic often increases growth, appetite, and activity of animals, corrects anemic conditions, and improves the coat and general appearance, and for these reasons arsenic was classed as a tonic. It is therefore not surprising that it was used by man for cosmetic purposes, especially for making the skin soft, the eyes bright, the hair lustrous, and the bust larger, and occasionally to increase the sexual powers. Indeed, in Austria and Germany preparations containing arsenic, called *Busenpillen*, were at one time commonly available. One of the most famous was the *Pilulæ Asiaticæ*, which contained arsenious acid, yeast extract, black pepper, and glycerol. Each pill contained 1 mg arsenious acid; it was advised that the largest single dose be 5 mg and the largest daily dose 10 mg arsenious acid. Groups of arsenic eaters have existed for centuries in certain mountainous regions of Austria, and it is used by them to increase strength, to ease respiration at the higher altitudes, and to prevent fatigue while hunting. Arsenical preparations have also been used as pubic depilatories by Mohammedan peoples. It is not known how much toxicity has resulted from such uses as these, nor is it known if there is a real basis for the beliefs in the efficacy of arsenic as a tonic or growth stimulator, since most of the early studies were poorly done and uncontrolled.* Sollmann (1921) gave rats 0.00005—0.005 mg/kg/day for 9—24 weeks and could detect no beneficial effects, only decreased appetite and weight being noted, but Von Tangl (1942) claimed some stimulatory effect of cuprisulfarsenite

* Arsanilic acid is marketed as a poultry growth stimulant under the name Pro-Gen by Abbott Laboratories and apparently its effectiveness has in this instance been demonstrated.
in rats and a large effect in pigs and fowl. Administration of sodium cacodylate (Liebesny and Vogl, 1923) and arsenite (Knell, 1936) in small doses to human subjects brought about no marked tonic effects, although some increase in erythrocyte and hemoglobin was noted. The question of efficacy of mechanism has never been satisfactorily settled. It has been commonly thought that arsenicals depress catabolic processes, thus shifting the balance toward assimilation, but such a mechanism has never been established. The history of the uses of arsenic by various peoples, especially in Austria, is a fascinating story and well told in the book "Arsenik" by Allesch (1959).

**Theory of the Capillaries as the Site for Arsenical Toxicity**

Arsenite has been thought by many workers to be one of the most potent and specific capillary poisons, and most, if not all, the toxic effects observed to be due to capillary dilatation and damage, and the consequent loss of fluid through them. Indeed, it has been postulated that the beneficial effects believed to result from low-dosage arsenic intake, as described above, are based on vasodilatation and a better supply of blood to the tissues. Certainly one of the most complex and confused problems in the field of the arsenicals is their action on the blood vessels, particularly the capillaries, and the role this may play in causing the symptoms and lesions long recognized as characteristic of poisoning. Since this has some bearing on many of the effects of the arsenicals, it will be necessary to determine as far as possible the nature of the vascular action.

There is no doubt that the arsenicals at sufficiently high levels can relax vascular smooth muscle, cause leakage of plasma into the tissues, and bring about a multiple hemorrhagic state; the pertinent question is whether such actions occur in the usual clinical or toxic doses. We have seen (page 713) that arsenicals commonly lower blood pressure following parenteral administration, and it has generally been assumed that this is primarily the result of vasodilatation, although cardiac depression and central vaso-motor inhibition have also been considered. Unterberger and Boehm (1874) claimed that arsenite causes a paralysis of the splanchnic vessels and that they are no longer responsive to nerve stimulation, and others have observed similar effects. Arsenite vasodilatation has been noted in isolated frog legs, rabbit ears, and rabbit kidneys, rather high concentrations being required to give significant changes (Tscherkess, 1926), but Lendle and Reinhardt (1931) could not repeat these results on the frog legs, some responding by dilatation and some by constriction at arsenite concentrations of 0.11–0.77 mM. Ellinger and Schmitt (1933) studied the mesenteric circulation of the frog and found that arsenite first accelerated and then slowed the blood flow. A visible change in the capillary walls was thought to occur during the slowing, and diapedesis was observed, so that a direct action on the vessel walls was assumed. No change in the vascular resistance of
the perfused cat limb with 1 mM arsenite could be detected by Hitchcock (1946). Oelkers (1936) in a rather thorough study could not obtain good evidence for a significant vasodilatation by arsenite in a variety of preparations, and argued that arsenite exerts most of its effects directly on the tissues. Hanna and McHugo (1960) recently demonstrated that injections into rabbits of 1 mg/kg sodium arsenite lead to an increase in capillary permeability, as measured by trypan blue staining of the internal organs, and confirmed the sensitivity of the splanchnic capillaries. Poisoning and death from the arsenicals can definitely occur without obvious vascular changes, and Butzengeiger (1940) reported circulatory disturbances in only 15 of 180 patients with chronic arsenic poisoning, gangrene of the extremities occurring in six. The results from direct studies of the capillary responses to the arsenicals have thus been at variance and more thorough and critical work must be done before definite statements can be made.

Magnus (1899) was the first to report the occurrence of edema following injections of arsenite. Dogs given subcutaneous arsenite over 4–9 days developed marked edema in various organs, and Magnus concluded that this originates from the damage to the capillaries. Bornstein and Budelmann (1930) rightly concluded that it is impossible to assign a site for the action in whole animal work and investigated isolated preparations, such as the dog leg, in which they claim edema is produced, although no controls are evident. Edema of the skin, especially in the head and neck region, has been noted several times; this could be due to a direct effect on the capillaries, a blockade of the sympathetics (although this usually does not cause edema), or release of some substance, such as histamine, for which there is no evidence. Even 5 mM arsenite solutions are not very irritant or inflammatory when applied to abraded skin, the tongue, or the eye (Heubner, 1925). The differentiation between direct and vascular actions was attempted by using the cornea, which is avascular, but Ellinger (1931) found irritation and Oelkers (1936) did not, so that these experiments did not settle the issue, which is not surprising considering the quality of the work.

The responses of the skin to the organic arsenicals are not as easy to interpret as has generally been assumed. Lewisite and related vesicants are irritant to the skin and mucous membranes, but the striking effects are due in part to the greater volatility and penetrability of these compounds relative to other arsenicals. Much of the work with lewisite on the skin has been done with either pure lewisite or fairly highly concentrated solutions or vapors; even in the metabolic work of Thompson (1946) the small amounts of lewisite applied to the skin correspond to concentrations of 0.25–0.75 mM, assuming homogeneous distribution. The ability of various phenylarsenoxides to induce local skin reactions upon intracutaneous injection varies quite markedly, this depending on the ring groups, only the hydroxy, amino, and unsubstituted phenylarsenoxides being potent
There seems to be a rough parallel between the local inflammatory activity and the systemic toxicity. It is difficult to understand the low potencies of some of these substances, unless it is a matter of lack of penetration into the cells. After World War I it was believed that vesicants such as lewisite are irritant because of the HCl released in the tissues by hydrolysis. During World War II the group at Oxford under Peters emphasized the reactivity of such arslenicals with SH enzymes, especially pyruvate oxidase, and it was indeed shown that lewisite inhibits pyruvate oxidation in the skin (Thompson, 1946), although there was little justification for concluding that a selective inhibition of pyruvate oxidase occurs, inasmuch as only succinate oxidation was examined in addition. At the present time it is generally accepted that vesication, at least that caused by arslenicals, is due to a biochemical lesion at the pyruvate oxidase level (Peters et al., 1946; Peters, 1955). However, tempting as the picture is, there is no direct evidence for it. When lewisite is applied to the skin and erythema results, it is not known if the action is primarily on the vessels or on the skin cells. Vasodilatation could be secondary and result from products released from damaged cells. Vesication is the result of accelerated escape of plasma through capillary beds and can result from various types of cellular damage. Certainly it appears that the keto acid oxidases are among the enzyme systems most sensitive to the arslenicals, but this is only circumstantial evidence that here is the site of the biochemical lesion in the production of vesication. Histological studies of the effects of lewisite on the skin by Cameron et al. (1946) indicate damage to the skin cells. The blister in human skin shows a roof of epidermis, but the epidermal cells are badly injured, having lost their structure and exhibiting degenerate nuclei. The dermal cells also are damaged, as are the endothelial cells of the blood vessels, which are often necrotic. Since these results were obtained by using pure lewisite droplets, little can be concluded relative to the specificity of action at lower concentrations, but

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<th>Phenylarsenoxide</th>
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<td>( p{-\text{NHCONH}_2} )</td>
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</tr>
<tr>
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</tr>
<tr>
<td>( m{-\text{CONH}_2} )</td>
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<tr>
<td>( 3{-\text{NH}_2{-4\text{-OH}}} )</td>
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</tr>
<tr>
<td>( p{-\text{NHCOCH}_3} )</td>
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<td>( p{-\text{OH}} )</td>
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<tr>
<td>Unsubstituted</td>
<td>0.12</td>
</tr>
<tr>
<td>( p{-\text{NH}_2} )</td>
<td>0.11</td>
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</table>
from work on other tissues it is likely that lewisite can injure essentially any type of cell. The HCl released from lewisite probably plays no role since lewisite oxide is equally effective.

Thus the important question as to the significance of vascular actions in arsenical toxicity must remain unanswered. It has been said to be reasonable for the blood vessels to be selectively affected because they are initially in contact with the highest concentrations of arsenical as it leaves the blood stream, but this applies to any inhibitor given in the same way, particularly the mercurials and heavy metals which are not generally considered capillary poisons. If the vascular smooth muscle is indeed especially susceptible to the arsenicals, this implies that they possess unique metabolic properties; if this is true, further work on this problem would be interesting.

Some Effects on the Metabolism in Whole Animals

Certain changes induced in nitrogen, lipid, and respiratory metabolism by the arsenicals, and the accumulation of keto acids in the blood, have been discussed in previous sections. We shall now take up various metabolic effects which may or may not have bearing on understanding the mechanisms by which the arsenicals exert their toxic actions. Several early workers found that arsenite causes a loss of glycogen from the liver; by administration of high enough doses, essentially all the glycogen can be depleted within 3–5 hr. It was further noted that there is sometimes little or no glucosuria accompanying this glycogen depletion. If glucose is given during arsenite poisoning, no glycogen is formed and glucose appears in the urine. Glycogen falls in other tissues but muscle glycogen is fairly resistant. We must note carefully the difference in the responses to large toxic doses and to the small chronic doses such as have been shown to increase weight. Delhougne (1934) reported that with very small doses (0.001–0.003 mg arsenite daily in the food), rats increase in weight and, in contrast to the above observations, the liver and muscle glycogen increase by 34% and 55%, respectively, the lipid and nitrogen contents not changing appreciably. We must thus entertain the possibility that small doses can reduce glycogen utilization, whereas larger doses can by some mechanism stimulate the breakdown of glycogen.

This situation can be better understood if we look at the changes in the blood glucose of arsenical-treated animals. Changes in the glucose level had been reported early, but the unreliability of the work and the variations in dosage and the arsenical used confused the picture. Almost 100 years ago it was reported that arsenical medication favorably influences the course of diabetes, but subsequent study did not confirm the ability of the arsenicals to prevent induced glucosuria. Van Dyke (1925) found that intravenous arsenite in doses considerably subtoxic (4 mg/kg) causes a distinct hyperglycemia in rabbits and that this occurs after bilateral splanchn-
notomy. He concluded that arsenite acts peripherally by increasing hepatic glycogenolysis. This hyperglycemia may be antagonized by insulin. Gruhzit (1935) reported that only fairly high doses of arsenite cause a rise of blood glucose in dogs and, indeed, Van Dyke had shown that dogs are less responsive than rabbits. Oelkers (1937) reinvestigated these matters more thoroughly and showed that arsenite at 6 mg/kg prolongs the blood glucose levels following injection of glucose (Fig. 6-7), the blood glucose taking 5–6 hr to return to normal and in untreated animals only 1.5–2 hr. A similar

![Fig. 6-7. Effects of arsenite on the blood glucose levels under various conditions. The dose was 6 mg/kg As₂O₃. The solid curves are controls and the dashed curves give the arsenite effects. (From Oelkers, 1937.)](image)

effect on epinephrine-induced hyperglycemia was observed and the hypoglycemic fall produced by insulin is antagonized by arsenite. Arsenite at near-toxic doses markedly elevates the blood glucose in normal animals; thus 5 mg/kg intravenously almost doubles the blood glucose after 3–4 hr (Oelkers, 1939). This work indicates a definite impairment by arsenite of the utilization of glucose in the tissues, and it is entirely possible that this can account for much of the glycogen loss. Berry and Smythe (1959) concur with this on the basis of their results in mice given 6.25 mg/kg arsenite intraperitoneally (see accompanying tabulation). It is difficult to understand the interference with glucose utilization since one might expect the opposite, and indeed work on isolated tissues has shown that arsenite often augments the uptake of glucose (page 676). The possibility must be considered that these changes are secondary to tissue damage
from these toxic doses of arsenite. It is interesting that arsenite simultaneously can triple liver citrate. Citrate levels in other tissues are increased more moderately (Berry et al., 1954b). What is the source of the citrate and why would it accumulate? Hallman (1940) has shown that arsenite is a potent inhibitor of citrate formation from a variety of substrates in kidney and testis, and a block of the cycle at the keto acid oxidases would lead one to expect a reduction in citrate. The answer to these and related problems must await more extensive investigation with wider dosage ranges, chronological correlations, and more detailed analyses.

### Mechanisms of Arsenical Toxicity

The possible mechanisms by which the arslenicals produce their effects on tissues and on whole animals will be discussed briefly to summarize our knowledge. The concept of arslenoreceptors in the cells as proposed by Ehrlich (1909) is valid and emphasizes the selectivity of action; it has been necessary to determine exactly what these receptors are. That these receptors involve SH groups was postulated by Ehrlich and put on a firm basis by Voegtlin et al. (1923), but the nature of these SH groups was not understood, so that Voegtlin et al. (1925) could state, “In fact, our data seem to indicate that the sulfhydryl group of glutathione actually represents the “arseno-receptor” of mammalian protoplasm.” However, as Brown and Kolmer (1929) soon showed, intensive arslenical therapy does not significantly reduce tissue glutathione (although they stated that the toxic effects may be related to glutathione depletion). Voegtlin admitted that other SH groups, perhaps fixed in the cells, could also be involved. The evolution of the concept of the selective action of the arslenicals on lipoate and keto acid oxidation has been described. The present generally accepted theory of the mechanism might be put somewhat as follows: arslenicals block specifically the oxidation of the α-keto acids, particularly pyruvate, by inactivation of the lipoate component of such systems, and thus interfere with the main oxidative pathways of the tricarboxylate cycle, the result
being a depletion of high-energy substances generated in the cycle and secondary alterations in various metabolic pathways associated with the cycle. We shall now examine this theory to determine if it fits the facts.

The fundamental evidence for this theory may be summarized as follows: (1) arsenicals depress oxidative processes in tissues both in vitro and in vivo; (2) the keto acid oxidases are very sensitive to the arsenicals; (3) accumulation of keto acids occurs in the tissues and blood of poisoned animals; and (4) the symptoms of arsenical poisoning are, at least partially, similar to those of thiamine deficiency, this also interfering with the oxidation of the keto acids. It is worthwhile to examine this last point in greater detail. Sexton and Gowdey (1947) presented a long table listing the changes in arsenical poisoning and thiamine deficiency, pointing out numerous similarities, although such similarities had been suggested much earlier. Their interest had been stimulated by the rapid recovery of a patient with arsenical encephalopathy and neuritis after administration of thiamine, and they showed that thiamine given to patients receiving oxophenarsine, and who exhibited high blood pyruvate levels, caused a fall in these levels, and that blood levels of pyruvate could be maintained at normal values by the simultaneous administration of thiamine and oxophenarsine to dogs. It is difficult to understand, if the lipoate is inactivated by the arsenicals, how the giving of thiamine could reverse this inhibition, and such has not been demonstrated on isolated systems or enzyme preparations. In comparing the symptoms of thiamine deficiency with arsenical poisoning, one must distinguish between acute and chronic effects, the acute toxicity perhaps not being pertinent in this regard. The classical symptoms of thiamine deficiency are: anorexia, weakness, loss of weight, peripheral neuritis, edema, gastrointestinal disturbances, and cardiac failure. Certainly this picture fairly closely parallels chronic arsenical poisoning, with the possible exception of the cardiac effects, which have not been studied adequately. However, the arsenicals can produce effects not seen in thiamine deficiency. The lack of an exact parallelism is not surprising because the biochemical lesion in the two cases is not identical. First, it is not certain that thiamine deficiency expresses itself solely as a depression of keto acid oxidation, or that arsenicals exert a perfectly specific effect on these enzyme systems. Second, the matter of penetration and distribution of the arsenicals is important; the relative effects on the various tissues would not necessarily be the same in the two situations inasmuch as some tissues might be relatively impermeable to the arsenicals and yet suffer readily from thiamine deficiency. Conversely, arsenicals may be concentrated in certain tissues, as the kidney, which might suffer more damage than in thiamine deficiency. There are, however, certain discrepancies to be explained; for example, the marked effects of the arsenicals on hematopoietic tissues, which is not paralleled exactly by thiamine deficiency, as well as the liver
changes. One may here, perhaps, call upon other actions of the arsenicals. It is probably not justifiable to conclude in acute poisoning, or with respect to the effects of relatively high arsenical concentrations on isolated tissue functions, that the mechanism is entirely a blockade of keto acid oxidation. It is really very difficult to prove that the inhibition of keto acid oxidation is the direct cause of any effect. However, all in all, the theory holds up well and is an important approach to arsenical toxicity. In the case of arsine, some of the pentavalent arsenicals, or the dissubstituted arsinoxides, other mechanisms are generally more important. Sandground and Hamilton (1943) found that p-aminobenzoate protects rats against atoxyl and carbarsone, relatively small doses of the protector being necessary; this has not been explained. It seems very questionable, however, whether this theory can be extended to other metals and vitamin deficiencies, as has been attempted by W. Hughes (1950).

**EFFECTS ON PROTOZOA**

Arsenicals are used clinically chiefly in the therapy of protozoan infections and much work has been done to elucidate the mechanisms of this inhibitory action. An excellent review of the historical aspects of the field may be found in the book by Work and Work (1948). A short time before 1900, Lingaard found that arsenite is of some value in surra, a trypanosomai disease of horses in India, and Bruce discovered that it causes a temporary remission of the tsetse fly disease in Africa. Ehrlich began work on human trypanosomiasis, for which no cure was known, around 1902, using dyes and then arsenicals. In 1903 he tested atoxyl against trypanosomes in vitro and found it to be inactive. However, Thomas and Breinl observed that atoxyl cures trypanosomiasis in mice, and Koch reported that preliminary work in Africa had demonstrated some efficacy in the human form of the disease. This revived Ehrlich’s interest and, upon finding its correct structure, he proceeded to synthesize numerous derivatives, this work leading to essentially all the known arsenicals and drugs of use in other infections. Most of the work aimed at discovering the mechanisms of action has been done on trypanosomes, rather than spirochaetes. It should be mentioned, however, that many types of protozoa have been found to be susceptible to the arsenicals: spirochaetes, such as *Treponema pallidum* (syphilis) and *Treponema pertenue* (yaws); trypanosomes of many species, including *Trypanosoma gambiense* and *Trypanosoma rhodesiense* (human sleeping sickness), and *Trypanosoma equiperdum* (equine trypanosomiasis); ciliates, such as *Paramecium*, *Colpidium*, and *Tetrahymena*; flagellates, such as *Trichomonas* and *Strigomonas*; and various species of *Entamoeba*, *Leishmania*, and *Balantium*. Different arsenicals are commonly used to treat infections by these agents; e. g., oxophenarsine for syphilis, tryparsamide
for trypanosomiasis, and carbarsone for amebiasis. Some workers believe the common denominator to be the trivalent arsenoxide forms, and the differences in potency and efficacy to be related to the factors of penetration and distribution. There is no question but that various protozoa vary markedly in susceptibility to different arsenicals in vitro, and that a change in the structure may increase activity toward one and decrease it toward another, but such can be explained on the basis of different membrane properties and degrees of penetration (whether it is true or not). One can say, at least, that most protozoa are more susceptible than other microorganisms to the arsenoxides. Our principal problem will be to determine as far as possible the mechanisms by which these arsenicals immobilize and kill protozoa.

Quantities of the Arsenicals Necessary for Inhibition

The concentrations of arsenicals required for immobilization or the killing of protozoa are often quite low, as is evident from Table 6-11 in which a few examples have been gathered to illustrate this potency. The time factor is of obvious importance; the concentration required to immobilize or kill in a short time being much greater than that required over many hours. This is probably due to the time taken for the organisms to accumulate sufficient arsenical, the actual amount of arsenical bound to the organism probably being roughly the same in all cases when immobilization or death occurs. These inhibitions also depend on the density of the trypanosome suspension, especially when low concentrations of the arsenicals are used, a high density protecting by distributing the available arsenical between many organisms, so that the behavior is quite similar to the pseudo-irreversible inhibition of enzymes.

Since 1930 it has been generally believed that the active trypanocidal arsenicals are bound to the trypanosomes while the inactive ones are not, and a large amount of evidence confirming this has been reported. The most convincing study was by Eagle and Magnuson (1944), who tested some 30 arsenical derivatives against trypanosomes, and their results have been plotted in Fig. 6-8 to show how the amount of arsenical bound is related to the relative trypanocidal activity. The ratio of the amount of arsenical bound to the trypanosomes to that in the supernate also rises with the activity in a similar manner, and for phenylarsenoxide is 224 under the experimental conditions. It is interesting now to consider how much arsenical must be bound to trypanosomes to exert a lethal action. Reiner et al. (1932) found that one trypanosome binds approximately $6 \times 10^6$ molecules of phenylarsenoxide. They calculated the surface area of a cell to be $10^{-6}$ cm$^2$ and the area of a phenylarsenoxide molecule to be $3 \times 10^{-14}$ cm$^2$; thus, assuming a closely packed monolayer of arsenical on the surface of the cell, $3 \times 10^7$ molecules could be adsorbed, which is around 5 times
### Table 6-11

**Potencies of Antiprotozoal Action**

<table>
<thead>
<tr>
<th>Arsenical</th>
<th>Effect</th>
<th>Concentration (mM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenite</td>
<td>Immobilization of trypanosomes in 75 min</td>
<td>0.013</td>
<td>Rosenthal and Voegtl (1930)</td>
</tr>
<tr>
<td></td>
<td>Moderate reduction in trypanosomal motility</td>
<td>0.04</td>
<td>Desowitz (1956)</td>
</tr>
<tr>
<td></td>
<td>Inhibition of growth of <em>Entamoeba histolytica</em></td>
<td>0.025</td>
<td>Yang (1959)</td>
</tr>
<tr>
<td>Oxophenarsine</td>
<td>50% Immobilization of spirochaetes in 2–5 hr</td>
<td>0.0042</td>
<td>Eagle (1939 b)</td>
</tr>
<tr>
<td></td>
<td>Killing of trypanosomes in 6 hr</td>
<td>0.00007</td>
<td>Eagle and Doak (1951)</td>
</tr>
<tr>
<td></td>
<td>Immobilization of trypanosomes in 1 hr</td>
<td>0.0031</td>
<td>Rosenthal and Voegtl (1930)</td>
</tr>
<tr>
<td></td>
<td>50% Inhibition of trypanosomal respiration</td>
<td>0.0043</td>
<td>Harvey (1949)</td>
</tr>
<tr>
<td>Phenylarsenoxide</td>
<td>Killing of trypanosomes in 6 hr</td>
<td>0.000018</td>
<td>King and Strangeways (1942)</td>
</tr>
<tr>
<td></td>
<td>Killing of trypanosomes in 24 hr</td>
<td>0.000014</td>
<td>Hawking (1938)</td>
</tr>
<tr>
<td>Acetarsone</td>
<td>Inhibition of growth of <em>Entamoeba gingivalis</em></td>
<td>0.023</td>
<td>Howitt (1926)</td>
</tr>
<tr>
<td>Atoxyl oxide</td>
<td>Killing of trypanosomes in 24 hr</td>
<td>0.000055</td>
<td>Fulton and Christophers (1938)</td>
</tr>
<tr>
<td></td>
<td>Inhibition of trypanosomal respiration</td>
<td>0.0055</td>
<td>Fulton and Christophers (1938)</td>
</tr>
<tr>
<td>Tryparsamide oxide</td>
<td>Killing of trypanosomes in 24 hr</td>
<td>0.000037</td>
<td>Fulton and Christophers (1938)</td>
</tr>
<tr>
<td></td>
<td>Inhibition of trypanosomal respiration</td>
<td>0.000037</td>
<td>Fulton and Christophers (1938)</td>
</tr>
<tr>
<td></td>
<td>Killing of trypanosomes in 24 hr</td>
<td>0.000022</td>
<td>Hawking (1938)</td>
</tr>
</tbody>
</table>
the value found. It is quite likely, however, that the binding is not just at the surface and that most of the arsenical is bound within the cell. These figures indicate that a great deal of arsenical is not required to kill these organisms and that specific sites are probably involved. It must be realized in such studies that all of the arsenical bound is probably not responsible for the lethal action and that these figures are undoubtedly higher than the true values for "active binding." Hawking (1938) found that under certain circumstances the ratio between trypanosome-bound arsenical

![Graph](image)

**Fig. 6-8.** Binding of arsenicals by trypanosomes as related to the trypanocidal activities. (From Eagle and Magnuson, 1944.)

and arsenical in the medium is 10,000 for phenylarsenoxide, 5000 for tryparsamide oxide, and 30–100 for arsenite. The amount of tryparsamide oxide required to kill in 3 hr is $8 \times 10^{-15}$ g per cell, which is about $10^7$ molecules per trypanosome, and this was calculated to be sufficient to cover about 5% of the cell surface; even less would be required for a lethal action in 24 hr. How do these values correspond to the number of SH groups in the trypanosomal cell? Harvey (1948) detected $2.7 \times 10^{-3}$ meq SH per g protein, or $1.6 \times 10^{-14}$ meq SH per cell in *Trypanosoma equiperdum*, and calculated that this would bind around $4.8 \times 10^6$ molecules of arsenical, a figure of the same order of magnitude as the experimental values given above. There are two difficulties in making such comparisons: (1) Although it is easy to determine the total SH + SS groups in cells, it is difficult to
establish what fraction is in the SH form under normal conditions, where certainly much is in the disulfide form in proteins; and (2) the average ratio arsenical/SH is uncertain, i. e., one does not know exactly the type of binding, whether it is mainly linear (arsenical/SH = 1) or cyclic (arsenical/SH = 0.5). The figures of Harvey are based on the latter assumption, which is probably correct. However, the amount of arsenical bound to functional SH groups (those whose inactivation contributes directly to the trypanocidal action), e. g., to lipoate, may be only a small fraction of this. It nevertheless seems to indicate that the bulk of the arsenical taken up or bound to these cells is attached to SH groups. It is assumed for the less active arsenicals, where less is bound, that less penetrates into the cells. The potent arsenicals would thus fall into the class of very active substances, those for which only a few million or less molecules need be bound to the cell for marked changes to be manifested, the amounts being similar to those for acetylcholine, histamine, and certain hormones. It is worth noting that the metabolic state of the cells is of some importance in the binding of the arsenicals, since Pedlow and Reiner (1935) found the addition of glucose to alter the amounts of oxophenarsine and neoarsphenamine bound to trypanosomes. No difference in binding was observed in vacuum or 100% oxygen, although the permeability of the cells might be changed as well as the number of functional SH groups available for reaction.

**Kinetics of Action**

When trypanosomes or spirochaetes are exposed to arsphenamine or pentavalent arsenicals, a fairly long lag period is observed, due in part to the time required for these arsenicals to be split and/or reduced. However, there is also a lag period when trivalent arsenicals are used in reasonable concentrations, and this lag period is longer than the period necessary for the taking up of sufficient arsenical to kill. The trivalent lag period is thus probably the sum of two parts: (1) the period for accumulation or binding of an adequate amount of arsenical, and (2) the time required for the death of the cells following inhibition of the enzyme systems involved. Trypanosomes begin to swell after a certain interval of exposure, become distorted, and eventually lyse (Hawking, 1938). The following equation was used:

\[(C - C_0)^n t = k\]

where \(C_0\) is the minimal concentration for an effect. The value of \(n\) was found to be 0.5–0.8 for arsenite and tryparsamide oxide, and 1.04 for phenylarsenoxide.* It was concluded that the total process consists of three

* An equation with a \((t - t_a)\) term might be somewhat better inasmuch as there is a minimal time for any concentration to produce a characteristic effect.
phases: (1) fixation of the arsenical, (2) secondary chemical reactions due to the fixation, and (3) the death process within the cells. The fixation occurs rapidly with the potent arsenicals, being complete within a few minutes, and is reversible. Neuschlosz (1919) used the same equation to describe the action of arsenite on paramecia, and determined the following values: $C_0 = 1 \text{ mM}, n = 1.38$, and $k = 1.21$.

A lag period for the pentavalent arsenicals is also observed in vivo. This and the reason are well illustrated by Murgatroyd et al. (1934), who injected tryparsamide and tryparsamide oxide intravenously into rabbits and determined the trypanocidal activity in the serum after various times (Fig. 6-9). The oxide is immediately active whereas tryparsamide exerts a maximal activity only after 6–8 hr indicating that tryparsamide must be reduced before it is effective. When animals with trypanosomiasis are treated with oxophenarsine, the trypanosomes rapidly disappear from the blood; e.g., 5 mg/kg intravenously brings about disappearance in 14–15 min (Pfeiffer and Tatum, 1935). That this is not entirely due to the killing and lysis of the trypanosomes was shown by removing a sample of the blood 5 min after the administration of the oxophenarsine, in which the organisms lived for 70–90 min. The disappearance of the cells from the blood stream in vivo must be in part due to the participation of the defense mechanisms of the host, particularly the reticuloendothelial system.
Relation of Arsenical Structure to Activity

As we have observed with regard to toxicity, very few substituent groups increase the parasiticidal activity of phenylarsenoxide and no marked increases occur at all; on the other hand, the activity is often greatly decreased. A summary of the results of Eagle and his collaborators is given in Table 6-12. Eagle and Doak (1951) divided the substituent groups into four general classes and the relative activities and parasiticidal/toxic ratios are averaged in the accompanying tabulation, with full realization that there is much variation within the groups. The inert groups include

<table>
<thead>
<tr>
<th>Groups</th>
<th>Relative molar activity</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Toxicity</td>
<td>Treponema</td>
</tr>
<tr>
<td>Inert</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>Slightly active</td>
<td>23</td>
<td>38</td>
</tr>
<tr>
<td>Acidic</td>
<td>16</td>
<td>4.7</td>
</tr>
<tr>
<td>Amides</td>
<td>7.2</td>
<td>30</td>
</tr>
</tbody>
</table>

\( \text{NO}_2, \text{CH}_3, \text{Cl}, \text{NH}_3, \text{OH}, \text{and F}; \) the slightly active include \( \text{RNH}_2, \text{ROH}, \text{NHCOCH}_3, \text{RNHCOCH}_3, \text{and COCH}_3; \) the acidic groups include \( \text{COOH, SO}_3\text{H, and ROOH}; \) and the amides include \( \text{CONH}_2, \text{RCONH}_2, \text{and SO}_2\text{NH}_2. \) The inert groups may slightly reduce the activity and toxicity but do not significantly alter the ratio, while the slightly active groups exert a moderate effect on both. The acidic groups quite markedly reduce all types of activity, but the parasiticidal more than the toxic, and hence the ratio falls, whereas the amides are relatively less toxic as shown by the definitely high ratios. Therapeutically one would, of course, desire a high ratio. As usual, there are exceptional cases; thus the 3-\( \text{NH}_2 \)-4-\( \text{OH} \)-\( \alpha \)-\( \text{AsO} \), or oxophenarsine, although containing two inert groups, not only exhibits a high ratio but is also clinically useful.

Derivatives with acidic groups form an interesting class, not only because of their marked reduction in activity, but also because of the surprising effects which are dependent on chain length. That activity is reduced is not surprising, since one might expect negatively charged molecules to penetrate less readily; this effect is more pronounced for the protozoa. Esterification of these acidic groups restores the activity toward normal as expected; however, the formation of an amide group has the odd effect of further reducing the toxicity while increasing the parasiticidal activity compared to the free acidic compounds. Differences in the permeability properties of host and parasite cells are evident here. The differences between the compounds with COOH groups on different positions of the ring can
### Table 6-12

**Relative Molar Activities of Substituted Phenylarsenoxides**

<table>
<thead>
<tr>
<th>Phenylarsenoxide</th>
<th>Relative molar activity</th>
<th>Ratio of parasiticidal to toxic activities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Toxicity</td>
<td>Treponema</td>
</tr>
<tr>
<td><strong>Acidic groups</strong></td>
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<td></td>
</tr>
<tr>
<td>o-COOH</td>
<td>27</td>
<td>28</td>
</tr>
<tr>
<td>m-COOH</td>
<td>16</td>
<td>13</td>
</tr>
<tr>
<td>p-COOH</td>
<td>41.4</td>
<td>6.7</td>
</tr>
<tr>
<td>p-CH₂COOH</td>
<td>41</td>
<td>4.2</td>
</tr>
<tr>
<td>p-CH₃CH₂COOH</td>
<td>7.3</td>
<td>4.1</td>
</tr>
<tr>
<td>p-CH₂CH₂CH₂COOH</td>
<td>8.8</td>
<td>1.9</td>
</tr>
<tr>
<td>p-CH₂CH₂CH₂CH₂COOH</td>
<td>7.4</td>
<td>—</td>
</tr>
<tr>
<td>p-CH₃CH₂CH₂CH₂CH₂COOH</td>
<td>8.1</td>
<td>22</td>
</tr>
<tr>
<td>p-CH=CHCOOH</td>
<td>9.4</td>
<td>17</td>
</tr>
<tr>
<td>p-CH(OH)COOH</td>
<td>7.0</td>
<td>2.8</td>
</tr>
<tr>
<td>p-OCH₃COOH</td>
<td>25</td>
<td>4.2</td>
</tr>
<tr>
<td>p-CONHCH₂COOH</td>
<td>15.6</td>
<td>0.7</td>
</tr>
<tr>
<td>p-NHCOCH₂CH₂COOH</td>
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<td>6.4</td>
</tr>
<tr>
<td>3-NO₂-4-COOH</td>
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<td>18</td>
</tr>
<tr>
<td>3-NH₂-4-COOH</td>
<td>15.1</td>
<td>20.1</td>
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<tr>
<td>O-SO₃H</td>
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<td>1.5</td>
</tr>
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<td>p-SO₃H</td>
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<td>3.3</td>
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Free amide groups

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<tr>
<th>Amide Group</th>
<th>Effect 1</th>
<th>Effect 2</th>
<th>Effect 3</th>
<th>Effect 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>m-CONH₂</td>
<td>9.8</td>
<td>41</td>
<td>39</td>
<td>4.2</td>
</tr>
<tr>
<td>p-CONH₂</td>
<td>9.6</td>
<td>45</td>
<td>45</td>
<td>4.7</td>
</tr>
<tr>
<td>p-CH₂CONH₂</td>
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<td>p-CH₂CH₂CH₂CONH₂</td>
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<tr>
<td>p-CH-CHCONH₂</td>
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<tr>
<td>p-CNHCONH₂</td>
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<td>34</td>
<td>5.3</td>
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<tr>
<td>p-CNHCH₂CONH₂</td>
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<td>15</td>
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<tr>
<td>p-CH₂CONHCH₂CONH₂</td>
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<td>11</td>
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<tr>
<td>p-SO₂NHCH₂CONH₂</td>
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<td>6.1</td>
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</tr>
<tr>
<td>p-SO₂NH₂</td>
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<td>29</td>
<td>24</td>
<td>6.0</td>
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Substituted amide groups

<table>
<thead>
<tr>
<th>Amide Group</th>
<th>Effect 1</th>
<th>Effect 2</th>
<th>Effect 3</th>
<th>Effect 4</th>
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<tbody>
<tr>
<td>p-CONHCH₃</td>
<td>15</td>
<td>54</td>
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<td>3.6</td>
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<tr>
<td>p-CON(CH₃)₂</td>
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<td>48</td>
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<td>2.5</td>
</tr>
<tr>
<td>p-CNHCH₂CH₃</td>
<td>26</td>
<td>59</td>
<td>—</td>
<td>2.3</td>
</tr>
<tr>
<td>p-CON(CH₂CH₃)₂</td>
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<tr>
<td>p-CNH-η</td>
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<td>97</td>
<td>—</td>
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<tr>
<td>p-CNHCH₂-η</td>
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<tr>
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<td>27</td>
<td>10.5</td>
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<td>p-SO₂N(CH₃)₂</td>
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<td>112</td>
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<tr>
<td>Phenylarsenoxide</td>
<td>Relative molar activity</td>
<td>Ratio of parasiticidal to toxic activities</td>
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<td>Toxicity</td>
<td>Treponema</td>
<td>Trypanosoma</td>
<td>Treponema</td>
</tr>
<tr>
<td>( p\text{-SO}_2\text{N(CH}_2\text{CH}_3)_2 )</td>
<td>134</td>
<td>101</td>
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<tr>
<td>( p\text{-SO}_2\text{NHCH}_2\text{CH}_2\text{OH} )</td>
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<td>Esters</td>
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<td>84</td>
<td>66</td>
<td>0.99</td>
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<tr>
<td>( m\text{-OH} )</td>
<td>49</td>
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<td>—</td>
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<td>72</td>
<td>—</td>
<td>1.5</td>
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<tr>
<td>( 3,4\text{-diOH} )</td>
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<td>65.7</td>
<td>—</td>
<td>0.59</td>
</tr>
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<tr>
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<td>51</td>
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<td>—</td>
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<td>Nitro groups</td>
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<td>m-No²</td>
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<td>89</td>
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<td>0.86</td>
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<td>2,4-diCl</td>
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<td>100</td>
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<td>0.59</td>
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<td>Methyl groups</td>
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<td>84</td>
<td>91</td>
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<tr>
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<td>97</td>
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<td>0.98</td>
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<td>102</td>
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<td>Relative molar activity</td>
<td>Ratio of parasiticidal to toxic activities</td>
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<td></td>
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<td>-------------------------</td>
<td>------------------------------------------</td>
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<td></td>
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<td>Treponema</td>
<td>Trypanosoma</td>
<td>Treponema</td>
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<td>Toxicity</td>
<td>Treponema</td>
<td>Trypanosoma</td>
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<td>Miscellaneous groups</td>
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<td>$p$-CO-$q$</td>
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<td></td>
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<td>$p$-OCH$_3$</td>
<td>118</td>
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<td>$p$-COCH$_3$</td>
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<td>$p$-CH$_3$NHOCH$_3$</td>
<td>51</td>
<td>78.5</td>
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<td>107</td>
<td>106</td>
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<tr>
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<td>71.1</td>
<td>—</td>
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<tr>
<td>3-NO$_2$-4-COOH</td>
<td>27</td>
<td>18</td>
<td>17.6</td>
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<tr>
<td>3-NO$_2$-4-SO$_2$H</td>
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<td>64.2</td>
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</tr>
<tr>
<td>3-NH$_2$-4-Cl</td>
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<td>98.7</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>3-NH$_2$-4-OCH$_3$</td>
<td>104</td>
<td>97.1</td>
<td>—</td>
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</tr>
<tr>
<td>3-NH$_2$-4-COOH</td>
<td>15.1</td>
<td>20.1</td>
<td>4.0</td>
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<td>3-NH$_2$-4-CONH$_2$</td>
<td>5.6</td>
<td>28</td>
<td>52</td>
<td></td>
</tr>
<tr>
<td>Compound</td>
<td>Dose</td>
<td>Activity</td>
<td>Unit</td>
<td>Relative Activity</td>
</tr>
<tr>
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<td>-------</td>
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</tr>
<tr>
<td>3-NH₂-4-CH₂CH₂OH</td>
<td>44.1</td>
<td>70</td>
<td>—</td>
<td>1.59</td>
</tr>
<tr>
<td>3-N(CH₃)₂-4-OH</td>
<td>75</td>
<td>57.2</td>
<td>—</td>
<td>0.77</td>
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<tr>
<td>3-NHCOCH₃-4-OH</td>
<td>12.5</td>
<td>24.1</td>
<td>3.0</td>
<td>1.93</td>
</tr>
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<td>3-OH-4-CONH₂</td>
<td>21</td>
<td>44.7</td>
<td>48</td>
<td>2.13</td>
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<td>3-Cl-4-OH</td>
<td>91.5</td>
<td>50.2</td>
<td>—</td>
<td>0.55</td>
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<tr>
<td>3,4-diNHCOCH₃</td>
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<td>7.95</td>
<td>—</td>
<td>1.37</td>
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<tr>
<td>3,5-diNH₂-4-OH</td>
<td>3.72</td>
<td>10</td>
<td>—</td>
<td>2.69</td>
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</table>

**Dithioarsenites**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>Activity</th>
<th>Unit</th>
<th>Relative Activity</th>
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<tr>
<td>Dicycisteinyl-o-CH₃</td>
<td>90</td>
<td>109</td>
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<td>1.21</td>
</tr>
<tr>
<td>Dicycisteinyl-m-COOH</td>
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<td>11</td>
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<td>0.73</td>
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<tr>
<td>Dicycisteinyl-p-SO₂H</td>
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<td>3.6</td>
<td>—</td>
<td>0.15</td>
</tr>
<tr>
<td>Dicycisteinyl-p-CONH₂</td>
<td>4.6</td>
<td>39</td>
<td>—</td>
<td>8.5</td>
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<tr>
<td>Dithiosalicyl-p-CONH₂</td>
<td>6.1</td>
<td>23</td>
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<tr>
<td>Dicycisteinyl-p-NHCONH₂</td>
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<td>7.24</td>
</tr>
<tr>
<td>Dicycisteinyl-p-OCH₂CONH₂</td>
<td>7.3</td>
<td>32</td>
<td>—</td>
<td>4.39</td>
</tr>
<tr>
<td>Dicycisteinyl-p-CONH₂CH₂CONH₂</td>
<td>1.6</td>
<td>17</td>
<td>—</td>
<td>10.6</td>
</tr>
<tr>
<td>Dicycisteinyl-p-CONH₂SO₂NH₂</td>
<td>3.3</td>
<td>22</td>
<td>—</td>
<td>6.67</td>
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<td>3.3</td>
<td>24</td>
<td>—</td>
<td>7.26</td>
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<tr>
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<td>4.1</td>
<td>29</td>
<td>—</td>
<td>7.07</td>
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*a The relative molar activities are based on 100 for phenylarsenoxyde. The species used are *Treponema pallidum* and *Trypanosoma equiperdum*. The toxicities are evaluated in mice by intraperitoneal injection. The data were obtained from Eagle (1945), Eagle and Doak (1951), and Eagle et al. (1940, 1942, 1943, 1944 a, b).
be partly explained by the effect of the As=O group on the $pK_a$ of the COOH group. It is noteworthy that the presence of these acidic groups does not alter the acidic properties of the As=O group. The interesting series, wherein the length of the chain bearing a terminal COOH group is increased, shows an unexpected rise in trypanocidal activity in the butyric and valeric derivatives, without simultaneous changes in the toxicity, a fact taken advantage of in developing compounds (e.g. butarsen) useful in trypanosomiasis. If the differences here are a matter of penetration, the permeability properties of these membranes are indeed unique. A further complication is the possibility that the acidic derivatives are not active themselves but are changed into active substances in the cells. The evidence for this is as follows (Eagle and Doak, 1951): (1) the rate of excretion of the acidic compounds is rapid at first but then levels off suddenly, indicating a change to some other type of substance; and (2) death of the animals is delayed relative to other derivatives. The low parasiticidal activity may be due to the fact that the protozoa cannot bring about this change, whereas the host tissues can. Both of these facts may be explained in other ways, however, since a slow penetration into cells with subsequent tight binding would account for the rapid initial excretion (as long as free arsenical remains) and the delayed lethal action (one might expect that the more readily a derivative penetrates, the faster would be the toxic or lethal action). Such results also argue against the simple assumption that the negative charge is the major factor in determining the activity here. It is true that a rough correlation may be made between activity and the $pK_a$ of the substituted group, but there are exceptions; e.g., the 3-NO$_2$-4-COOH derivative is more acidic and the activity is greater (see page 777 for the effects of pH). It is interesting also that the effect of adding an NH$_2$ group adjacent to the COOH group largely cancels the effect of the acidic group. This might be explained on the basis of the amphoteric nature of the disubstituted derivative and an over-all neutral charge at physiological pH, or of the reduction in acidity of the COOH group induced by the vicinal NH$_3^+$ group; however, this will not explain the decreased toxicity so readily.

The polysubstituted derivatives present some interesting and unexpected behavior. No general additivity rules can be formulated; i.e., from the effects of the single substituents it is impossible to predict accurately the activity of the disubstituted derivative. Furthermore, it is not just a matter of the groups themselves but of their relative positions on the ring, as may be seen in the case of the aminophenols and aminobenzoates. Even where only one type of group is involved the effect cannot be predicted, as in the 3,4-diOH derivative, which is even more toxic than phenylarsenoxide, despite the fact that both the 3-OH and 4-OH derivatives are much less so. It is true that certain rough rules may be formulated, e.g., the addition of
a butyric acid group influences favorably the parasiticidal/toxic ratio, as in 3-NH₂-4-CH₂CH₂CH₂COOH, inert substituents generally do not markedly alter the properties when added to another group, and the addition of an acidic group generally reduces the activity and the ratio. However, when the problem presumably centers around complex mechanisms of membrane penetration, it would be surprising if predictable behavior were observed.

The problem of why the terminal amide group so favors the parasiticidal/toxic ratio (or reduces the host toxicity so specifically) cannot at the present time be answered. One can say only that, if it is a question of penetration, the protozoal membranes allow the passage of the amides much more readily than do mammalian tissue cells, in comparison with the unsubstituted phenylarsenoxide. It is certainly not the size of the group alone, since the length of the side chain is of relatively little importance here, nor is it merely the amide group, since many of the substituted amide exhibit the same properties (it appears that the —CONH— or —CON= group is the determinant). Furthermore, the sulfonamides behave very similarly.

The thioarsenites also present an interesting group. These will be discussed in a later section, but it may be noted now that the formation of a dithioarsenite lowers somewhat both the toxicity and the parasiticidal activity, the former more than the latter, so that the ratio increases. Summarizing the ten dicysteiny1 derivatives in Table 6-12, one can calculate that the toxicity is reduced 33%, the treponemicidal activity reduced 14%, and the ratio increased 38%, although there are exceptions. It is evident that these results cannot be explained by the simple hydrolysis of the dithioarsenites to the corresponding derivatives. Anderson et al. (1949) have shown that two dithioarsenites derived from carbarsone oxide are antiamebic but with reduced toxicity, and similar work has brought about the clinical use of certain dithioarsenites.

**Effective Trypanocidal Doses**

Doses effective in clearing the blood of mice of trypanosomes and lethal doses in mice are shown in Table 6-13. If one may presume the right to average such variant values, one obtains the figures in the accompanying tabulation which give a rough idea of the relationships. These figures show

<table>
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<tr>
<th></th>
<th>Trivalent</th>
<th>Pentavalent</th>
<th>Ratio</th>
<th>As (V)/As (III)</th>
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<td>0.15</td>
<td>8.90</td>
<td>59</td>
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<td>ED₅₀</td>
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<td>3.02</td>
<td>220</td>
<td></td>
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<tr>
<td>LD₅₀/ED₅₀</td>
<td>10.9</td>
<td>2.95</td>
<td>0.27</td>
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<td>Arsenical&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Trypanocidal activity</td>
<td>LD&lt;sub&gt;50&lt;/sub&gt;/ED&lt;sub&gt;50&lt;/sub&gt;</td>
<td></td>
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<td>------------------------</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>LD&lt;sub&gt;50&lt;/sub&gt;</td>
<td>ED&lt;sub&gt;50&lt;/sub&gt;</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>millimoles/kg</td>
<td>millimoles/kg</td>
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</tr>
<tr>
<td></td>
<td>As (III)</td>
<td>As (V)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ratio</td>
<td>Ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p-NH&lt;sub&gt;2&lt;/sub&gt;</td>
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<tr>
<td>p-SO&lt;sub&gt;2&lt;/sub&gt;NH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.24</td>
<td>7.93</td>
<td>33</td>
<td>0.013</td>
</tr>
<tr>
<td>3-NH&lt;sub&gt;2&lt;/sub&gt;-4-OH</td>
<td>0.23</td>
<td>10.3</td>
<td>46</td>
<td>0.0083</td>
</tr>
<tr>
<td>3-NH&lt;sub&gt;2&lt;/sub&gt;-4-CONH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.20</td>
<td>3.87</td>
<td>19</td>
<td>—</td>
</tr>
<tr>
<td>p-CONHCH&lt;sub&gt;2&lt;/sub&gt;CONH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.20</td>
<td>23.4</td>
<td>111</td>
<td>0.019</td>
</tr>
<tr>
<td>p-NHCONH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.15</td>
<td>13.7</td>
<td>93</td>
<td>0.015</td>
</tr>
<tr>
<td>p-OCH&lt;sub&gt;3&lt;/sub&gt;CONH&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.13</td>
<td>9.93</td>
<td>78</td>
<td>0.011</td>
</tr>
<tr>
<td>3-NHCONH&lt;sub&gt;2&lt;/sub&gt;-4-OH</td>
<td>0.08</td>
<td>10.2</td>
<td>127</td>
<td>0.025</td>
</tr>
<tr>
<td>3-NH&lt;sub&gt;2&lt;/sub&gt;-4-CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;COOH</td>
<td>0.16</td>
<td>1.87</td>
<td>12</td>
<td>0.017</td>
</tr>
<tr>
<td>Melarsen</td>
<td>0.16</td>
<td>3.47</td>
<td>22</td>
<td>0.00064</td>
</tr>
<tr>
<td>p-OH</td>
<td>0.054</td>
<td>2.87</td>
<td>53</td>
<td>0.01</td>
</tr>
<tr>
<td>4-Glycine</td>
<td>0.35</td>
<td>9.31</td>
<td>27</td>
<td>0.087</td>
</tr>
<tr>
<td>Arscetin</td>
<td>0.076</td>
<td>8.80</td>
<td>116</td>
<td>0.0051</td>
</tr>
</tbody>
</table>

<sup>a</sup> The data in upper half of the table from Eagle and Doak (1951) and in the lower half from Yorke et al. (1931 a).

<sup>b</sup> These are all phenylarsenoxide or phenylarsonate derivatives.
well the marked differences in potency between the tri- and pentavalent arsenicals, and further indicate the LD$_{50}$/ED$_{50}$ ratio to be generally higher for the trivalents. In human therapy other factors play a role, since in these mouse experiments only the acute effects and blood clearance were determined. There is some variability in the LD$_{50}$/ED$_{50}$ ratio, but not nearly as much as in either dosage, confirming the concept that the toxic and trypanocidal potencies run fairly parallel. It should be noted that effective concentrations of the trivalent arsenicals in the body must be quite low. Of course, different species of trypanosome vary in susceptibility to the arsenicals (Kuhs and Tatum, 1937; Eagle and Doak, 1951). Arsenicals having acidic aliphatic side chains and curing Trypanosoma lewisi infections in rats may be ineffective against Trypanosoma equiperdum, for which derivatives with basic side chains are more effective.

**Effects on Protozoa Other Than Spirochaetes and Trypanosomes**

Most protozoa are not as sensitive to the arsenicals as are the trypanosomes and spirochaetes, as may be seen from the data in Table 6-14, although in certain instances the organic arsinoxides are quite potent. There is no reason, of course, for expecting so diversified a group as the protozoa to respond in a manner even remotely uniform; one knows that various types of protozoa have quite different metabolic patterns. Two investigations of the relation of structure to action have been made and the results are presented in Table 6-15. One fact stands out clearly: the high potency of the disubstituted arsenicals, particularly when it is considered that a lethal action within 3 min is the end-point. It is also evident that aromatic derivatives are more potent than aliphatic ones. One might imagine that the action is unrelated to the arsenic group, but four pentavalents tested on Colpidium gave relative activities of 6–16, so that, unless permeability to these is low, some specific attack by the diphenyl derivatives appears to be implied. It would be very interesting to know what enzyme and metabolic disturbances are produced by these dissubstituted arsenicals, and if monothiol enzymes are indeed involved. Further investigation of the actions of these arsenicals on ciliates and other protozoa at lower concentrations and over longer periods of time would be worthwhile, since it might be more important to know the changes when motility is depressed or growth is reduced. The only information we have is the effects of arsenite on Paramaecium: 0.1 mM slows the activity, inhibits acetate utilization 90%, depresses respiration 60%, and increases glucose utilization 15%, all of which fits in with the standard picture of action on keto acid oxidation (Holland and Humphrey, 1953). The motility of Tetrahymena is also reduced at 0.1 mM arsenite, and this is followed by cytological changes progressing to death; the cytoplasm becomes granular, the macronucleus swells and degenerative spheres appear in it, the nuclear composition is
## Table 6-14

### Some Effects of the Arsenicals on Various Protozoa

<table>
<thead>
<tr>
<th>Arsenical</th>
<th>Organism</th>
<th>Effect</th>
<th>Concentration (mM)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenite</td>
<td><em>Paramecium caudatum</em></td>
<td>Inhibition of motility</td>
<td>0.0013</td>
<td>Heubner (1925)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Killing in 30 min</td>
<td>19</td>
<td>Neuschlosz (1919)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Killing in 24 hr</td>
<td>1.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Killing in 48 hr</td>
<td>1</td>
<td>Jollos (1921)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibition of motility and respiration</td>
<td>0.1</td>
<td>Holland and Humphrey (1953)</td>
</tr>
<tr>
<td></td>
<td><em>Tetrahymena pyriformis</em></td>
<td>Killing in 2 hr</td>
<td>2.5</td>
<td>Neuhaus (1910)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibition of motility and death</td>
<td>0.1</td>
<td>Chatton and Tellier (1934)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>62% Inhibition of respiration</td>
<td>10</td>
<td>Ryley (1952)</td>
</tr>
<tr>
<td><em>Plasmodium berghei</em></td>
<td>60% Inhibition of respiration</td>
<td></td>
<td>10</td>
<td>Fulton and Spooner (1956 a)</td>
</tr>
<tr>
<td><em>Trichomonas foetus</em></td>
<td>22% Inhibition of fermentation</td>
<td></td>
<td>1.2</td>
<td>Ryley (1955 b)</td>
</tr>
<tr>
<td></td>
<td><em>Navicula pelliculosa</em></td>
<td>Inhibition of motility</td>
<td>0.1</td>
<td>Lewin (1954, 1955)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhibition of respiration and silicon uptake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phenylarsenoxide</td>
<td><em>Paramecium caudatum</em></td>
<td>Killing in 3 min</td>
<td>0.36</td>
<td>McCleland and Peters (1919)</td>
</tr>
<tr>
<td></td>
<td><em>Tetrahymena scintillans</em></td>
<td>Killing in 3 min</td>
<td>0.036</td>
<td>Walker (1928)</td>
</tr>
<tr>
<td>Oxophenarsine</td>
<td><em>Entamoeba histolytica</em></td>
<td>Killing in 48 hr</td>
<td>0.14-0.21</td>
<td>Anderson and Chuan (1944)</td>
</tr>
<tr>
<td></td>
<td><em>Colpidoium colpoda</em></td>
<td>Death</td>
<td>0.7-1</td>
<td>Peters and Stocken (1947)</td>
</tr>
<tr>
<td>Carbarsone oxide</td>
<td><em>Entamoeba histolytica</em></td>
<td>Killing in 48 hr</td>
<td>0.011-0.1</td>
<td>Anderson et al. (1949)</td>
</tr>
<tr>
<td>Diphenylchloroarsine</td>
<td><em>Tetrahymena scintillans</em></td>
<td>Killing in 3 min</td>
<td>0.009</td>
<td>Walker (1928)</td>
</tr>
</tbody>
</table>
### Table 6-15

**Arsenical Concentrations Lethal to Ciliates**

<table>
<thead>
<tr>
<th>Arsenical</th>
<th>Lethal concentration&lt;sup&gt;b&lt;/sup&gt; (mM)</th>
<th>Relative activity&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Paramecium caudatum</strong>&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Di&lt;sub&gt;4&lt;/sub&gt;q-AsOAs-di&lt;sub&gt;4&lt;/sub&gt;q</td>
<td>0.02</td>
<td>1800</td>
</tr>
<tr>
<td>Di&lt;sub&gt;4&lt;/sub&gt;q-AsCl</td>
<td>0.07</td>
<td>514</td>
</tr>
<tr>
<td>q-AsO</td>
<td>0.36</td>
<td>100</td>
</tr>
<tr>
<td>Et-chlorovinyl-AsCl</td>
<td>0.45</td>
<td>80</td>
</tr>
<tr>
<td>p-Cl-q-AsCl</td>
<td>0.47</td>
<td>77</td>
</tr>
<tr>
<td>DiMe-AsOAs-diMe</td>
<td>0.85</td>
<td>42</td>
</tr>
<tr>
<td>q-AsCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.89</td>
<td>41</td>
</tr>
<tr>
<td>DiMe-AsCl</td>
<td>1.4</td>
<td>26</td>
</tr>
<tr>
<td>Et-AsO</td>
<td>4.6</td>
<td>7.8</td>
</tr>
<tr>
<td>Et-AsBr&lt;sub&gt;2&lt;/sub&gt;</td>
<td>7.0</td>
<td>5.1</td>
</tr>
<tr>
<td>Me-AsCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>7.9</td>
<td>4.6</td>
</tr>
<tr>
<td>Et-AsCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>14.3</td>
<td>2.5</td>
</tr>
<tr>
<td><strong>Colpidium colpoda</strong>&lt;sup&gt;e&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Di&lt;sub&gt;4&lt;/sub&gt;q-AsBr</td>
<td>0.0044</td>
<td>2480</td>
</tr>
<tr>
<td>Di&lt;sub&gt;4&lt;/sub&gt;q-AsOAs-di&lt;sub&gt;4&lt;/sub&gt;q</td>
<td>0.0045</td>
<td>2420</td>
</tr>
<tr>
<td>Di&lt;sub&gt;4&lt;/sub&gt;q-AsCl</td>
<td>0.0056</td>
<td>1950</td>
</tr>
<tr>
<td>q-AsO</td>
<td>0.109</td>
<td>100</td>
</tr>
<tr>
<td>p-Cl-q-AsCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.303</td>
<td>36</td>
</tr>
<tr>
<td>DiMe-AsCl</td>
<td>0.443</td>
<td>25</td>
</tr>
<tr>
<td>Di&lt;sub&gt;4&lt;/sub&gt;q-As-sulfide</td>
<td>0.475</td>
<td>23</td>
</tr>
<tr>
<td>Me-AsCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.500</td>
<td>22</td>
</tr>
<tr>
<td>DiMe-AsOAs-diMe</td>
<td>0.645</td>
<td>17</td>
</tr>
</tbody>
</table>

<sup>a</sup> The terminologies are a little irregular and I am not certain that all the compounds have been correctly designated; e.g., I do not know what diphenylarsenious oxide is — perhaps it is di<sub>4</sub>q-AsOAs-di<sub>4</sub>q, which splits readily in aqueous medium.

<sup>b</sup> The concentration required to kill the organisms in 3 min.

<sup>c</sup> Relative activity based on 100 for phenylarsenoxide; for *C. colpoda* the value for this compound is maximal.

<sup>d</sup> From McCleland and Peters (1919).

<sup>e</sup> From Walker (1928).
altered (based on staining), and the cells finally agglutinate and lyse (Chatton and Tellier, 1934). There is little else to help us understand the mechanisms by which the arsenicals act on these protozoa.

**Mechanism of Trypanocidal Action**

There is little more to be discussed in regard to the mechanisms of toxicity, since it seems to be generally accepted that the trypanosomes are inactivated and killed because of a block in keto acid oxidation, but there is no proof of this. Inhibition of respiration and accumulation of pyruvate in trypanosomes have been demonstrated at low arsenical concentrations; e.g., respiration is depressed 50% by atoxyl oxide, tryparsamide oxide, oxophenarsine, and other arsenoxides in the concentration range 0.005–0.05 mM (Fulton and Christophers, 1938; Harvey, 1949), but still these concentrations are 10–100 times higher than those for trypanocidal action. There is no doubt that trypanosomes possess respiratory systems very sensitive to the arsenicals, and even arsenite inhibits well at 0.02 mM (Desowitz, 1956), other SH reagents being much less potent. There should be more study of the metabolism of organisms removed from arsenical-treated animals, since one could relate any changes observed to the state of the trypanosomes. This was done by Cantrell (1951, 1953), as we have discussed before, and his results are quite at variance with all work done in vitro. The glucose utilization is not altered, while actually somewhat less pyruvate is present, a result very difficult to explain by any known action of the arsenicals. In fact, from the known nature of trypanosomal metabolism, it is rather unexpected that the arsenicals depress so readily. The blood forms of most trypanosomes do not oxidize pyruvate, have no pyruvate oxidase activity in extracts, and do not even utilize succinate efficiently. In some (Trypanosoma vivax and Trypanosoma rhodesiense) the respiration is not inhibited by cyanide and there is no detectable cytochrome system. The R. Q. is often around 0.1–0.2 for the oxidation of glucose and most trypanosomes aerobically convert glucose to various organic acids, such as pyruvate, succinate, acetate, formate, and others. *Trypanosoma equiperdum*, upon which so much experimental work with the arsenicals has been done, almost quantitatively converts glucose to pyruvate under aerobic conditions, the cycle being absent. Spirochaetes also are known to grow better under anaerobic or semianaerobic conditions, and oxygen is in a way toxic to them. The problem then comes up as to the system with which the arsenicals react. If lipoate is the target substance, what is its function in these protozoa? Or are other dithiol components the sites of attack? Such matters seem to have been mainly ignored in discussing the mechanisms of the trypanocidal action, and there is really no evidence at all as to how the arsenicals act on these organisms.

Protection and reversal by thiols have been claimed to support the
hypothesis that arsenicals act by the usually accepted mechanism. Protection data have little or no bearing on how the arsenicals, or other inhibitors, act, as has been clearly stated by Eagle and Doak (1951) and emphasized repeatedly in the present work. Let us see if any useful information can be obtained from the studies on recovery. The pertinent results may be summarized as follows: (1) cysteine added to a suspension of spirochaetes being progressively inactivated by phenylarsenoxide immediately stops the action of the arsenical, and the number of motile organisms remains relatively constant (Eagle, 1940); (2) trypanosomes immobilized by phenylarsenoxide can be resuscitated by cysteine and dimercaprol, and simultaneously the arsenical is partially withdrawn from the cells (Eagle et al., 1946); and (3) dimercaprol is able to halt and reverse the trypanocidal action of various arsenicals in vivo. The results with dimercaprol indicate that it binds arsenicals more tightly than the trypanosomal groups, since essentially complete reactivation can be obtained if the dimercaprol is added within 5–10 min (phenylarsenoxide was used at 0.013 mJ and this completely immobilizes within 1 min and begins to cause degenerative changes by 5 min). If dimercaprol is added 15 min or longer after the arsenical, progressively less reversal is observed, which is not surprising since a good deal of lysis takes place after 15 min. It may seem rather remarkable that cysteine is effective, but very high molar ratios with respect to the arsenical must be used; e.g., ratios of cysteine/phenylarsenoxide greater than 625 were quite effective in an experiment of Eagle et al. (1946), but at a ratio of 312 there is no reversal at all, even if the cysteine is added immediately after the arsenical. Dimercaprol is effective at a molar ratio of 3 or above. Nevertheless, the fact that cysteine can reverse the immobilization and withdraw the arsenicals from the trypanosomes may possibly indicate that the arsenicals do not inhibit here by reaction with lipoate since, in systems in which lipoate is the site of attack, cysteine is generally unable to reverse. It is not so much a question of whether the arsenicals combine with SH groups to produce their trypanocidal effects — actually from what we know there is no other possibility — but of the exact nature of the cellular thiols reacted. Reversal data are frequently suggestive but unless obtained from experiments carefully designed to provide relative binding affinities, they must be interpreted cautiously. For example, in the work of Eagle et al. (1946), although the trypanosome/medium ratio of arsenical was reduced from 154 to 33 by cysteine (molar ratio of 455), we note that there is still much arsenical in the cells, and the experiments with dimercaprol are similar in that the trypanosomes after reversal contain 10–30 times as much arsenical as the supernate. Does this mean that the more weakly bound fraction of the cellular arsenical is responsible for the immobilization? It seems that resuscitated trypanosomes can still contain a good deal of arsenical, although the experiments on reactivation
and arsenical withdrawal are not exactly comparable. It is impossible to answer such questions with the information we have but they must be considered in the formulation of experimental programs and theories of mechanism. The difficulty of establishing a mechanism in organisms whose metabolism is poorly understood, and in which the motility and mitotic processes are almost completely unknown, cannot be overestimated.

**EFFECTS ON BACTERIA, FUNGI, YEASTS, AND VIRUSES**

Since 1860 it has been known that arsenite inhibits putrefaction, and by 1900 it was realized that some bacteria are sensitive and some are not; e.g., streptococci and the cholera and typhoid bacilli are killed by relatively low concentrations, whereas staphylococci, *Escherichia coli*, and *Proteus* species grow fairly well in higher concentrations. *E. coli* will actually grow in 75 mM arsenite, according to early work. Yeasts and fungi are relatively resistant to arsenite; it may require 20 mM to kill yeast cells, although this depends on the density of the cell suspension, and *Penicillium brevicaule* grows in 15 mM arsenite, while various other molds have long been known to be capable of growing in Fowler’s solution. We shall see later that many fungi have the ability to metabolize arsenicals readily.

**Bacteria**

Only a very small amount of work has been done on the antibacterial activity of the arsenicals, inasmuch as attention has been directed mainly at the protozoa. We can find, for example, that arsenite inhibits the growth of *Thiobacillus thiooxidans* at 0.1 mM, without altering sulfur oxidation or respiration (Vogler et al., 1942), that 10 mM arsenite completely inhibits the growth of *Bacillus subtilis*, although there is no effect on the germination of spores (Hachisuka et al., 1955), that more than 2 mM arsenite is required to abolish the growth of *Proteus vulgaris* (Kandler et al., 1956), and similar isolated bits of data, but in no case has a thorough or comparative study been made, and only in isolated instances have the organic arsenicals been used. The only really interesting results were published by Albert et al. (1944), who reasoned that if the mercurials inhibit bacteria by acting on SH groups, as Fildes had postulated, arsenicals should also be bacteriostatic; they were surprised to find no references to such work on the organic arsenicals earlier than 1942, when Hirsch (1942) demonstrated inhibition of *E. coli* with atoxyl (which is probably unrelated to SH groups since the action is antagonized by p-aminobenzoate), and Osgood (1942) reported that neoarsphenamine can inhibit the growth of *Streptococcus viridans* in bone marrow culture after many hours. Albert and his co-workers determined the concentrations which just inhibit growth (see
accompanying tabulation), and these data show that (1) arsenicals are as potent as HgCl₂ against certain bacteria, (2) *E. coli* and *P. vulgaris* are fairly resistant to the arsenicals, but not to HgCl₂, confirming earlier observations, and (3) the pentavalent arsenicals are not active. Inhibition would certainly be expected in bacteria utilizing the cycle and obtaining energy and synthetic units from pyruvate and related compounds, but again the matter of penetration must be considered. It is very interesting that 3 mM arsenite does not alter the growth of *Aerobacter aerogenes*, either aerobically or anaerobically, but still causes the accumulation of pyruvate (anaerobically from 24 to 360) and certain amino acids (Fowler and Werkmann, 1955). Arsenite injected into mice at 5.4 mg/kg increases somewhat the mortality of animals infected with various bacteria (Berry and Mitchell, 1953 c; Berry *et al.*, 1954 a), and infection with *Salmonella* produces rises in tissue citrate similar to those seen in arsenite-treated animals (Berry *et al.*, 1954 b). Thus the cycle was assumed to play a role in the susceptibility of the host, and the elevated mortality perhaps to be due to summated cycle blocking, although the effects of arsenite were rather minimal, especially compared to malonate.

### Yeasts and Fungi

The early studies indicated the relative resistance of yeast to arsenite, 10 mM inhibiting growth moderately, but complete inhibition occurring only at 20 mM (Johannsohn, 1874). A stimulation of growth at lower concentrations was often noted (Diemair and Schülke, 1941). There is some evidence that the penetration of arsenicals into yeast does not readily occur, although reaction with the surface leads to a change in the isoelectric point of the cells (Janke and Garzuly-Janke, 1936). The growth and sporulation of yeast are inhibited equally by arsenite (Miller and Halpern, 1956). Arsenite at 1 mM fairly well inhibits the growth of some species of *Candida* and *Torula*, but *Torula utilis* is resistant (Nickerson and Van Rij, 1949). Pyruvate oxidation in *Penicillium chrysogenum* is inhibited
only 65% by 10 mM arsenite (Hockenhull et al., 1954 b) and incubation with arsenite leads to the accumulation mainly of $\alpha$-ketoglutarate rather than pyruvate, indicating a somewhat different pattern of metabolism from that in most organisms or tissues (Hockenhull et al., 1951). Although 1 mM arsenite interferes with the formation of itaconate from glucose in *Aspergillus terreus*, mycelial weights increased almost 2-fold, perhaps showing a diversion of pyruvate into cell material (Bentley and Thiessen, 1957). Only spore formation and not growth is blocked by arsenite in *Aspergillus niger*, which is quite different from the results with most bacteria. The sporulation of *Venturia inequalis* to produce infectious conidia is likewise specifically inhibited by arsenite at 10–100 mM with respect to growth, although the respiration is inhibited (Kirkham and Flood, 1963). The germination of *Neurospora* spores is 50% reduced by 1 mM arsenite and completely by 10 mM (Sussman et al., 1958). The only conclusion that can be drawn from these heterogeneous observations, and the metabolic effects discussed in other sections, is that the yeasts and fungi are quite resistant to arsenicals, possibly due in part to the failure to penetrate adequately into the cells.

**Viruses**

The multiplication of *E. coli* phage is inhibited completely by 0.6 mM arsenite (Spizizen, 1943) and this effect is specific in the sense that phage multiplication can be suppressed without a marked bacteriostatic action (Dolby, 1955). Arsenite is one of the few inhibitors tested which possesses this selectivity. It inhibits phage growth completely when added 15 min after the latent period has begun, and partially inhibits after 22 min, indicating a critical period for the effect. Oxophenarsine at 0.04 mM is also capable of inhibiting coliphage multiplication without altering the growth of the host cells (Czekalowski, 1952). However, at 0.4 mM it acts on both phage and bacteria. Arsenite at 0.38 mM inhibits the proliferation of *Corynebacterium diphtheriae* phage, but can convert an avirulent phage to a virulent one (Hewitt, 1954). In one case, arsenite was effective in ridding the bacteria of one of the lysogenic virus strains. The yield of foot-and-mouth disease virus in cultures of pig kidney cells is reduced 90% by 0.05 mM arsenite, and completely by 0.1 mM, and this inhibition is not reversed by the addition of succinate or glutamate (Planterose, 1961). There is thus some evidence that arsenicals in the proper concentration range can selectively suppress virus or phage proliferation, and it seems that this should stimulate further work in the field, not only for the purpose of finding the mechanism by which the arsenicals act but also to obtain information on the factors involved in virus multiplication.
RESISTANCE TO THE ARSENICALS

That certain organisms have the ability to develop a high degree of tolerance during exposure to the arsénicals has been recognized for over 50 years, inasmuch as the earliest work on the trypanocidal action of atoxyl in Ehrlich's laboratory (Franke and Röhl, 1907) demonstrated recurrences in infected animals in which the drug had lost its effectiveness. Resistance* was first obtained experimentally by Browning (1907), on leave from Glasgow and working in Frankfurt, who found that definite tolerance can be developed within 10 days in infected mice and that this resistance is maintained, in one case after 140 passages through mice over 14 months. Ehrlich (1909) pointed out the specificity of resistance — that trypanosomes resistant to one arsénical may not be resistant to others, or that organisms resistant to other compounds, such as dyes, are not necessarily resistant to the arsénicals — and explained this on the basis of his arsénobacter receptor theory. In this connection it is worth pointing out, as did Yorke and Murgatroyd (1930), that the term "arsénical resistance" is a misnomer inasmuch as a nonspecific general tolerance to all arsénicals does not seem to develop, but only resistance to certain classes of arsénicals. It also has long been known that certain microorganisms possess a natural resistance; fungi such as Cladosporium, Rhizopus, and Aspergillus can grow in Fowler's solution, which is approximately 50 mM arsenite, unless other preservatives are added. The growth of bacteria in arsénical cattle-dipping fluid was first reported from Queensland in 1909 but the bacteria were not isolated until 1918, and only recently has a study of this been made (Turner, 1954), showing that species of Pseudomonas, Xanthomonas, and Achromobacter can grow in 20–40 mM arsenite and furthermore oxidize the arsenite, although it is unknown whether they can derive energy from this process. Resistance to the arsénicals is not only of practical interest but is a phenomenon which may possibly shed some light on the mechanisms of arsénical action since, as is the case with any drug tolerance, a study of the changes occurring in these organisms may relate directly to the vulnerable systems in the normal organisms.

Resistance to the arsénicals has been demonstrated in a variety of bacteria, fungi, and protozoa. Some organisms, or strains, are apparently naturally resistant and others achieve tolerance upon exposure to the arsénicals. Aside from the trypanosomes, upon which the most thorough work has been done, acquired resistance has been demonstrated in spirochaetes (Treponema and Borrelia), bacteria (Escherichia and Salmonella), infusorium (Paramecium and Colpidium), and the South African blue tick (Boophilus).

* In this section we shall be discussing acquired tolerance or resistance unless it is otherwise specified.
Tolerance has also been induced in the higher animals but this will be considered separately, inasmuch as different problems are involved.

**Conditions under Which Resistance May Develop**

An excellent discussion of the techniques used to induce resistance in microorganisms is given by Schnitzer and Grunberg (1957), in which the reader may find a more detailed account than is necessary here. In general, procedures may be classified as to whether exposure is *in vitro* or *in vivo*. Before trypanosomes could be grown in cultures, *in vitro* exposure was carried out during an interval between passages, so that contact with the arsenical was made only during a nonproliferative stage. Several exposures of only 1-hr duration each led to a strain at least 500-fold more resistant than the original strain to tryparsamide oxide (Yorke *et al.*., 1931 c). Continuous exposure of organisms growing in liquid media to increasing concentrations of arsenicals often leads to quite marked tolerance. Resistance can be achieved surprisingly easily *in vivo*. For example, in a single infected animal a high degree of resistance may be produced by successive arsenical administrations; even a single exposure is occasionally effective, although the resistance is not great. One of the most effective techniques is the “relapse method,” in which sufficient arsenical is administered to clear the blood of trypanosomes, and when new ones appear they are transferred to other animals, this procedure continuing as long as necessary.

**Degree, Rate of Onset, and Duration of Resistance**

The *resistance factor*, the ratio of the arsenical dosage or concentration necessary to immobilize or kill a tolerant strain to that required for the normal strain, may vary from 2 to over 1000, depending on the arsenical used, the organism, and the conditions of the exposure (a few examples are given in Table 6-16). The rate at which resistance develops varies, of course, with many factors but it is interesting that different arsenicals are apparently able to induce resistance at various rates. Results obtained with *Trypanosoma rhodesiense* are shown in the accompanying tabulation.

<table>
<thead>
<tr>
<th>Arsenical</th>
<th>Time for maximal resistance (days)</th>
<th>Resistance factor (tryparsamide oxide)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atoxyl</td>
<td>37</td>
<td>256</td>
</tr>
<tr>
<td>Arsacetin</td>
<td>62</td>
<td>96</td>
</tr>
<tr>
<td>Tryparsamide</td>
<td>33</td>
<td>256</td>
</tr>
<tr>
<td>Dichlorophenarsine</td>
<td>132</td>
<td>256</td>
</tr>
<tr>
<td>Arsenophenylglycine</td>
<td>316</td>
<td>4</td>
</tr>
</tbody>
</table>
Table 6-16
EXAMPLES OF ACQUIRED RESISTANCE TO THE ARSENICALS

<table>
<thead>
<tr>
<th>Organism</th>
<th>Arsenical</th>
<th>Resistance factor</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Escherichia coli</em></td>
<td>Oxophenarsine</td>
<td>10</td>
<td>Akiba and Ishii (1952)</td>
</tr>
<tr>
<td></td>
<td>Dichlorophenarsine</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td><em>Paramecium caudatum</em></td>
<td>Arsenite</td>
<td>2</td>
<td>Jollos (1921)</td>
</tr>
<tr>
<td></td>
<td>Arsenite</td>
<td>4</td>
<td>Neuschlosz (1920)</td>
</tr>
<tr>
<td><em>Borrelia gallinae</em></td>
<td>Arsphenamine</td>
<td>10</td>
<td>Gonder (1912)</td>
</tr>
<tr>
<td><em>Trypanosoma equiperdum</em></td>
<td>Butarsen</td>
<td>24</td>
<td>Schumacher and Schnitzer (1956)</td>
</tr>
<tr>
<td><em>Trypanosoma gambiense</em></td>
<td>Tryparsamide</td>
<td>600</td>
<td>Tobie and von Brand (1953)</td>
</tr>
<tr>
<td><em>Trypanosoma rhodesiense</em></td>
<td>Dichlorophenarsine</td>
<td>67</td>
<td>Yorke <em>et al.</em> (1931 e)</td>
</tr>
<tr>
<td></td>
<td>Tryparsamide</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Tryparsamide oxide</td>
<td>968</td>
<td></td>
</tr>
</tbody>
</table>

(Yorke *et al.*, 1932). In some cases definite resistance has occurred following a single exposure to an arsentic and in others it has required many weeks or months. Spontaneous development of resistance in *Trypanosoma equiperdum* without exposure to an arsentic was reported by Eagle and Magnuson (1944). Over a period of 2-3 months the resistance of this strain increased progressively until a resistance factor as high as 200 was observed. The duration of the resistance varies to some extent with the degree of resistance; i.e., when only brief exposure to the arsentic results in a small degree of tolerance, this is apt to be lost rather readily, whereas strains of high resistance maintain their tolerance for very long times. An atoxyl-resistant strain of *Trypanosoma rhodesiense* was observed for 12.5 years and through 1528 passages in mice without alteration of the degree or specificity of resistance (Fulton and Yorke, 1941), and other instances of maintenance over periods of several months or years have been reported. The resistance in certain instances may possibly be considered as permanent (Voegtlind *et al.*, 1924). Resistance to arsenite in paramecia is lost very slowly, the rate depending, for one thing, on whether the organisms grow vegetatively or conjugation is allowed to occur (Jollos, 1921). In any event, it is quite clear that resistance often implies a change in the organism which is hereditary in the sense that it is maintained through many generations in the absence of the arsentic.
Ability of Different Arsenicals to Induce Resistance

It has been known from the time of Ehrlich that resistance is much easier to produce with some arsenicals than with others. In general it appears that pentavalent arsenicals, such as atoxyl and tryparsamide, induce resistance most readily, certain trivalent arsenicals, such as oxophenarsine, dichlorophenarsine, and butarsen, are intermediate, while arsenophenylglycine and arsenite induce resistance only with difficulty. No rapid or marked resistance has ever been produced with arsenite in any organism, and in many cases even prolonged exposure has resulted in failure to demonstrate tolerance, as in the exposure of Trypanosoma gambiense to arsenite over 13 months and during 40 passages (Tobie and von Brand, 1954). Organisms resistant to various arsenicals are usually susceptible to arsenophenylglycine, and it has been very difficult to induce resistance to this compound; the only instance is that in which a resistance factor of 4 was reached after 10 months of exposure by the relapse method (Yorke et al., 1932). Such behavior might imply basic differences in the mechanism of action of the various arsenicals, but we shall see that it may also be explained on the basis of different modes of penetration.

Cross-Resistance

If all the arsenicals penetrated and acted in the same manner, and if there is some common basic change in the resistant forms, one would expect cross-resistance to be complete. In fact, the earliest observations in Ehrlich’s laboratory showed that this is not the case and all recent work has confirmed this. An organism made resistant to one arsenical may or may not be resistant to another arsenical, or will generally show a degree of resistance either greater or less than to the inducing compound. It is interesting that strains made resistant to certain nonarsenical substances may show resistance to the arsenicals or, conversely, that arsenical-resistant strains may be resistant to nonarsenicals. Occasionally, for example, arsenical-resistant strains may be resistant to p-rosaniline; however, p-rosaniline-resistant strains have never been found to be resistant to the arsenicals. Typical arsenical-resistant organisms are usually not resistant to other trypanocidal agents, such as the diamidines, quinolines, or suramin, but may be resistant to the acridine dyes of the acriflavine type. Yet organisms made resistant to Melarsen exhibit resistance to the diamidines and other compounds, indicating that the resistance here may be unrelated to the arsenic group, or reaction with SH groups (Williamson and Rollo, 1959). A stilbamidine-resistant strain of Trypanosoma rhodesiense showed a resistance factor of 64 with Melarsen oxide, although the trypanocidal concentrations of numerous inhibitors were not altered (Williamson, 1959 b). It may also be noted that organisms resistant to some arsenicals are generally resistant to the antimonials.
Trypanosomes resistant to the usual arsenicals (e.g., atoxyl, tryparsamide, oxophenarsine, etc.) are generally resistant to many other arsenicals but to different degrees. Two examples of such cross-resistance will be given for illustration. The accompanying tabulation shows the concentrations necessary to kill trypanosomes in 6 hr (Yorke and Murgatroyd, 1930). An acriflavine-resistant strain showed the same pattern of resistances to the arsenicals as the atoxyl-resistant strain. The tabulation below shows the minimal effective doses subcutaneously to clear the blood of mice of trypanosomes (Schumacher and Schnitzer, 1956). A strain made resistant to butarsen showed essentially the same pattern, but was much more resistant to butarsen itself (resistance factor > 18). It has generally been found that strains resistant to atoxyl are not resistant to phenylarsenoxide or the inert-substituted derivatives, whereas some increase is

<table>
<thead>
<tr>
<th>Arsenical</th>
<th>Concentration (mM)</th>
<th>Resistance factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normals</td>
<td>Atoxyl-resistants</td>
</tr>
<tr>
<td>Dichlorophenarsine</td>
<td>0.00015</td>
<td>0.0049</td>
</tr>
<tr>
<td>Arsacetin oxide</td>
<td>0.00017</td>
<td>0.0055</td>
</tr>
<tr>
<td>Atoxyl oxide</td>
<td>0.00011</td>
<td>0.00086</td>
</tr>
<tr>
<td>Atoxyl</td>
<td>16</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Arsacetin</td>
<td>17.5</td>
<td>35</td>
</tr>
<tr>
<td>Tryparsamide</td>
<td>16.5</td>
<td>33</td>
</tr>
<tr>
<td>Arsenophenylglycine</td>
<td>0.006</td>
<td>0.012</td>
</tr>
<tr>
<td>Acetarsone</td>
<td>8.3</td>
<td>11.0</td>
</tr>
<tr>
<td>Arsenite</td>
<td>0.0048</td>
<td>0.0048</td>
</tr>
<tr>
<td>“Acriflavine”</td>
<td>0.0025</td>
<td>0.02</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>Minimal clearing dose (mg/kg)</th>
<th>Resistance factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normals</td>
<td>Tryparsamide-resistants</td>
</tr>
<tr>
<td>Tryparsamide</td>
<td>125</td>
<td>&gt;2000</td>
</tr>
<tr>
<td>Oxophenarsine</td>
<td>1</td>
<td>&gt;20</td>
</tr>
<tr>
<td>Neoarsphenamine</td>
<td>50</td>
<td>&gt;165</td>
</tr>
<tr>
<td>Stilbamidine</td>
<td>25</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Butarsen</td>
<td>1.4</td>
<td>2.7</td>
</tr>
<tr>
<td>“Acriflavine”</td>
<td>16.6</td>
<td>&gt;16.6</td>
</tr>
<tr>
<td>p-Rosaniline</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Trypan blue</td>
<td>500</td>
<td>500</td>
</tr>
</tbody>
</table>
seen in the resistance to the amino and hydroxy derivatives and good resistance to the amide derivatives (King and Strangeways, 1942). It is interesting to compare the susceptibilities of the spontaneously resistant strain of Eagle and Magnuson (1944) (see accompanying tabulation).

<table>
<thead>
<tr>
<th>Phenylarsenoxide</th>
<th>Resistance factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-NH₂-4-CO NH₂</td>
<td>205</td>
</tr>
<tr>
<td>p-SO₂NH₂</td>
<td>165</td>
</tr>
<tr>
<td>p-CO NH₂</td>
<td>140</td>
</tr>
<tr>
<td>3-NH₂-4-OCH₂CH₂OH</td>
<td>120</td>
</tr>
<tr>
<td>p-CH₃NHCOCH₃</td>
<td>88</td>
</tr>
<tr>
<td>p-NHCONH₂</td>
<td>57</td>
</tr>
<tr>
<td>p-CO NHCH₂CN</td>
<td>49</td>
</tr>
<tr>
<td>p-NHCOCH₃</td>
<td>38</td>
</tr>
<tr>
<td>3-NH₂-4-OH</td>
<td>35</td>
</tr>
<tr>
<td>m-CO NH₂</td>
<td>23</td>
</tr>
<tr>
<td>p-CO NHCONH₂</td>
<td>13</td>
</tr>
<tr>
<td>p-CH₃CH₂CH₂CO NH₂</td>
<td>7</td>
</tr>
<tr>
<td>p-NH₂</td>
<td>4.8</td>
</tr>
<tr>
<td>p-CH₃</td>
<td>1.3</td>
</tr>
<tr>
<td>p-CH₂CH₂CH₂COOH</td>
<td>1</td>
</tr>
<tr>
<td>α-Cl</td>
<td>0.8</td>
</tr>
<tr>
<td>3-NH₂-4-COOH</td>
<td>0.7</td>
</tr>
</tbody>
</table>

The resistance of this strain is thus primarily against the amide derivatives. The implications of such behavior with regard to the mechanism of action will be dealt with in a later section (page 765).

Characteristics of Arsenical-Resistant Strains

Following Ehrlich’s suggestion that there is a loss of the specific receptors in resistant organisms, one would like to know if resistsants have an altered thiol content, and particulary if there is a change in the functional SH groups. Voegtlin et al. (1924) felt that SH-containing receptors do not disappear but that a shift from SH to SS groups occurs, so that the ability to react with the arsenicals is reduced. They applied this particularly to glutathione. Harvey (1948) was the first to test this hypothesis experimentally by determining the SH and SS groups in normal and resistant trypansomes (see accompanying tabulation). There are perhaps differences but they cannot be correlated with resistance, and Harvey preferred to interpret these changes as secondary to the altered cell metabolism; in fact,
it was stated that the differences in the SH contents are not statistically significant. It may be noted that the number of SH groups per cell corresponds quite closely to the number of arsenical molecules bound per cell in

<table>
<thead>
<tr>
<th>Organism</th>
<th>Strain</th>
<th>Cell content (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SH groups</td>
</tr>
<tr>
<td><em>T. equiperdum</em></td>
<td>Normal</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>0.40</td>
</tr>
<tr>
<td><em>T. hippocum</em></td>
<td>Normal</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td>Resistant</td>
<td>0.27</td>
</tr>
</tbody>
</table>

the normal strain, but we shall see that much less arsenical is bound in the resistant forms. Akiba and Ishii (1952) found that a resistant strain of *E. coli* had 40–50% fewer SH groups than normals, but this could not account for the degree of resistance. Indeed, it is difficult by any modification of thiols to explain resistance factors of 100–1000. Arsenite-resistant blue ticks contain about twice as many SH groups as normal ticks, but it is not known if this is directly related to the resistance (Thompson and Johnston, 1958).

If the arsenicals are trypanocidal through interference with metabolism, we might expect to find the metabolism of resistant trypanosomes to be altered. It has been known for some time that certain metabolic systems of tolerant organisms cannot be readily depressed by arsenicals in the intact cell. Thus the suppression of glucose utilization in trypanosomes by oxophenarsine runs quite parallel to the sensitivity of the organism to the arsenical (Schueler et al., 1947). Harvey (1949) showed that it requires about 10 times as much oxophenarsine to inhibit the respiration of resistant trypanosomes compared to normal ones, although inhibitions by other substances are the same in both. Such work does not necessarily indicate differences in the enzymes or metabolic systems, since the arsenical may not gain access to these systems because of permeability changes in the resistant forms. In general, the over-all metabolism of normals and resi-

dants has been found to be the same. Some differences were claimed by Williamson (1953), using methylene blue reduction with a variety of substrates. The strain was made resistant to Melarsen and was also resistant to the pentavalent arsenicals and the diaminidines. No differences were seen with glucose, ethanol, or formate, and only slight differences with malate, but with the other six substrates there were marked differences (see accompanying tabulation). This appears to indicate that either (1) the metabolism of these cells was altered, or (2) the permeabilities to these substrates
were changed (since the work was done with cell suspensions). Williamson felt that triose utilization bypassed the 3-phosphoglyceraldehyde dehydrogenase step, which he assumed to be sensitive to the arsenicals; also that the disappearance of the pyruvate system might indicate a shift of the metabolism. Reliable interpretation cannot be made until quantitative work is carried out on enzymes obtained from normals and resistsants.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Dye reduction rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normals</td>
</tr>
<tr>
<td>Succinate</td>
<td>48</td>
</tr>
<tr>
<td>Glutamate</td>
<td>43</td>
</tr>
<tr>
<td>Glycerol</td>
<td>20</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>16</td>
</tr>
<tr>
<td>Lactate</td>
<td>0</td>
</tr>
<tr>
<td>Citrate</td>
<td>57</td>
</tr>
</tbody>
</table>

We may next inquire if there are changes in the biological properties of resistant organisms. Marks (1910) produced the first arsenical-resistant bacteria by culturing *Salmonella* with arsenite, and found that they become immotile, acquire a thicker shape, lose some flagella, and are altered in certain growth and culture characteristics, such as color. Resistant organisms show no particular change in virulence (Voegtlin *et al.*, 1924). However, some change in the immune response of the host was noted by Tobie and von Brand (1953), in that animals infected with a resistant strain survive significantly longer than with a normal strain, the survival time often being doubled. A change in agglutination properties also occurs; an oxophenarsine-resistant trypanosome showed a marked tendency to agglutinate when infected blood was diluted with physiological saline, although the cells remained motile (Cantrell, 1955). However, some other highly resistant strains were nonagglutinating, so that the two properties do not necessarily go together.

**Resistance in Higher Animals**

Ignoring the Styrian arsenic eaters, whose problems are not directly pertinent to our subject, one finds that a low degree of tolerance may be produced occasionally in the higher animals, but many failures have also been reported. Morishima (1900 a) could not induce resistance to arsenite in rabbits following multiple administrations, and Bucher (1950) was unable to show resistance in tissue cultures after 4 months of treatment with arsenite. It should be noted that arsenite has been used in most mammalian work and this form of arsenic has never been found to produce
significant resistance in microorganisms. Kuhs et al. (1939) succeeded in producing tolerance to the organic arsenicals in rats, rabbits, and dogs, but did not succeed with arsenite; furthermore, the animals which were resistant to oxophenarsine, tryparsamide, or carbarsone were not resistant to arsenite. The resistance factors were only 1.2–3 so that tolerance was slight although definite. Five to eight intravenous injections weekly over several weeks were required to induce this degree of tolerance, the dosage being increased slightly during this time. Norris and Elliott (1945) subjected rats to daily intraperitoneal injections of arsenite at levels not depressing growth or causing toxicity; at the end of 3 weeks, the dosage could be raised significantly (to levels normally toxic in 1 hr) and the characteristic hypothermia no longer occurred. It was proved that the tolerance is not due to alteration in the absorption from the peritoneal region, and it is known that the fraction of arsenical excreted by the kidney in the tolerant animal is the same as in normals. Bunting and Longley (1940) reasoned that since the kidneys suffer arsenical damage, some change might be observable in resistant animals. Rats were given increasing intravenous doses of tryparsamide for a month, at which time they were receiving 50% more than the dose just tolerated by normal animals. The kidneys initially showed a coagulative type of necrosis, especially in the convoluted tubules; regeneration occurred and, by the fifth week after the highest dose, there was no observable necrosis and the tubules were lined with mature epithelial cells. These regenerated cells are thus quite resistant to tryparsamide inasmuch as high doses no longer produce evidence of necrosis.

Mechanism of the Resistance

The resistance could develop as a direct adaptation to the presence of the arsenical, or could be the result of selection from an initial heterogeneous population, or could appear by selection of mutants arising during the exposure. The first explanation is the most likely but over long periods selection may also play a role. At least there are experiments which seem to exclude selection in some cases. For example, arsphenamine-resistant trypanosomes have been derived from a single cell, excluding selection from an original varying population; furthermore, resistance can be induced when exposure is only during periods when no growth or division is occurring (bound arsenical might, of course, be carried into the growth phase). The marked rapidity of the development of resistance in some cases also argues for a direct adaptation. In the development of high resistance, however, selection may occur, as indicated by Schueler et al. (1947), who found the sensitivity distribution curves for low-resistance and high-resistance strains not to overlap. The ability of low-resistance strains to revert to normal, whereas high-resistance strains seem very stable also points to some selection. The rate at which resistant organisms lose their resistance
has really very little to do with the mechanism of the resistance, especially in microorganisms. To call such phenomena "Dauermodifikations" or "permanent modifications" or "lingering modifications" does not help to explain the mechanism.

It is more important for us to determine why the resistant forms can withstand such relatively high concentrations of the arsenicals. The possible explanations might be classified under the following headings:

(A) Decreased penetration of the arsenicals into the cells
(B) Decreased affinity of the cells or their receptors for the arsenicals
(C) Increased destruction of the arsenicals
(D) Alteration of the metabolic pathways so that vulnerable components are no longer necessary

We have mentioned that no change in trypanosomal SH groups has been found which would account for the high degrees of resistance; if such did occur, it would certainly result in a modification of metabolism inasmuch as these SH groups should be functional in enzyme reactions. If there were a decrease in the receptor sites, it would probably be secondary to a primary change in the metabolism. It is difficult to conceive of an increased inactivation of the arsenical being responsible for the tolerance and there is no good evidence that such occurs; in fact, it has generally been observed that organisms resistant to high concentrations of arsenite are not capable of metabolizing it. Neuschlosz (1920) thought that an increased ability to oxidize arsenite might explain resistance in paramecia, and Turner (1954) considered this with regard to bacteria growing in cattle dips, but found some of the tolerant forms not to oxidize arsenite. The last possibility has been discussed and we have concluded that there are insufficient data; in certain instances metabolism may be altered but whether this is primary or secondary we do not know. Let us then consider the evidence for the first explanation based on altered permeability.

Early work had indicated that trypanosomes made resistant to dyes do not take up the dye from solution, and it was soon found that certain strains resistant to the arsenicals, and showing cross-resistance to dyes, also do not become stained when exposed to the dyes. Jollos (1921) in his experiments with arsenite-resistant paramecia analyzed filtrates from the media in which the paramecia had been exposed to arsenite, and found that the resistant ones take up less arsenite. Yorke et al. (1931 b) examined tryparsamide-resistant trypanosomes and showed that they do not take up appreciable amounts of tryparsamide oxide, whereas normals completely remove it from the medium; they postulated that resistance is basically due to the inability of the arsenical to penetrate into the resistant forms. These results were confirmed by Hawking (1937), who also showed that
arsenite, to which an atoxyl-resistant strain showed no resistance, is taken up equally and poorly by both normals and resistsants. Eagle and Magnuson (1944) found the same relations in their spontaneously resistant strain, and the correlation between trypanocidal activity and uptake is shown in Fig. 6-8. This type of experiment has been criticized on the basis that normal susceptible strains would be killed by the arsenaical, whereas resistsants would not, and that dead trypanosomes probably take up more of the arsenaical. Hawking (1937) showed that living and dead trypanosomes take up roughly the same amounts of arsenaical, and Eagle and Magnuson (1944) pointed out that their uptake studies were in the period before even motility was affected and hence there was no question of dead organisms.

It is thus certain that resistant organisms take up less arsenaicals than normal strains. The problem is whether this is a matter of altered membrane permeability or altered binding sites on or in the cells. Some have favored the former explanation (King and Strangeways, 1942; Eagle and Magnuson, 1944) and some the latter (Pedlow and Reiner, 1935; Hawking, 1937; Schueler, 1947), but in no case has clear evidence been produced on either side. If we assume that the arsenaicals are bound to cellular SH groups, a change in binding would imply either a disappearance of SH groups or a modification of their environment so as to make them unavailable for the arsenaicals; as we have noted, there is evidence that the former is not correct. In connection with the latter explanation, the work of Schueler (1947) is pertinent. Trypanosomes resistant to amino- or amide-substituted phenylarsenoxides show resistance to other basic arsenaicals but not to acidic or neutral derivatives; thus the development of resistance might be more related to these substituent groups than to the phenylarsenoxide nucleus. This is confirmed by the fact that organisms highly resistant to the various substituted phenylarsenoxides show little or no resistance to phenylarsenoxide itself. However, resistance to the acidic arsenaicals (e.g. butarsen) certainly implies no great specificity and we have seen that a butarsen-resistant strain is identical to a tryparsamide-resistant strain, except that the former is resistant to butarsen. Schueler postulated that the development of resistance might be associated with a change in the isoelectric point of the trypanosomal proteins; if, for example, an organism were resistant to a basic arsenaical (+ charge) it would be expected to have its isoelectric point shifted upward, so that there would be more + charges to repel the basic arsenaicals and prevent them from combining with the SH groups. If such did occur, differences in staining with acidic and basic dyes should be demonstrable and Schueler showed some differences with methylene blue and basic toluidine blue, these differences particularly involving granules within the cells. When these trypanosomes are first treated with oxophenarsine, the stainability of the normals is decreased, whereas the resistsants are not affected; treatment with butarsen, against which the or-
ganism is not resistant, depresses staining in the resitants. The difficulty is, however, that we are confronted by the same problem as previously: Is it binding or penetration which is altered? The above results could be explained in either way.

The following is perhaps the most likely explanation from the available data. There are two quite different stages or processes in the trypanocidal action of an arsenical: (1) the penetration of the arsenical into the cell, and (2) the reaction of the arsenical with SH groups within the cell. Each process is controlled by different factors. The penetration is not primarily dependent on the phenylarsenoxide nucleus but on the substituent groups (i.e., their size, distribution, and charge), while the intracellular reactions are dependent on the arsenoxide group and only secondarily on the substituents (there is no evidence of major differences between the phenylarsenoxides with regard to enzyme inhibition). The fact that acriflavine-resistant and atoxyl-resistant strains are similar also indicates that the resistance is not specifically related to the arsenoxide portion of the molecule. When trypanosomes become resistant to a particular type of arsenical, a change occurs in the membrane whereby the transmembrane passage of that arsenical and all those penetrating in the same manner is impeded, whereas those arsenicals penetrating by different routes are unaffected by the changes which have taken place and enter as readily as previously; the response to the unsubstituted neutral phenylarsenoxide would thus be unaffected.

Resistance in these organisms would then be primarily a matter of membrane changes and alterations in permeability. What these changes are one does not know but it may well be modification of charged groups within the membrane, particularly as changes in dye penetration occur simultaneously. Williamson and Rollo (1959) pointed out that cross-resistance behavior cannot be explained entirely on an ionic basis, and that the results suggest stereospecific structural changes associated with the initial arsenical uptake. It is also possible that the mechanisms of penetration involve systems which are normally operative in the uptake of metabolites, so that the metabolism might be altered secondarily. It must be emphasized that the bulk of this work has been done with trypanosomes, and we do not know if similar changes occur in other microorganisms or in those cases of resistance in the higher animals. The information obtained from the studies of resistance has provided very interesting concepts of arsenical penetration, but has not added a great deal to our knowledge of the biochemical mechanisms by which the arsenicals act within the cells. It has also made evident that, in comparing the relative potencies of a series of arsenicals, the differences cannot be safely interpreted in terms of differential effects on enzyme systems but rather may be related to differential penetration only, and the practical problem of finding a safe arsenical has been mainly one of finding molecules which will penetrate host cells relatively less readily than the parasite cells.
EFFECTS ON GROWTH AND MITOSIS

The often controversial effects of the arsenicals on animal growth have been briefly mentioned, but it is difficult to interpret such results in terms of cellular growth or proliferation since many other factors are involved. There is no doubt that toxic doses of the arsenicals reduce total growth and can depress mitosis in certain tissues. A generalized inhibition of the hematopoietic system can occur, the arsenicals interfering with the formation of erythrocytes and leucocytes, and causing the appearance of abnormal cells and mitotic disturbances. Depression of thrombocytes and granulocytes may be noted. These effects will be discussed in greater detail in the following section with regard to the treatment of leukemias. We shall therefore restrict our attention for the present to results on isolated cells and tissues.

Many tissues are susceptible to the antimitotic action of the arsenicals, but some microorganisms, as we have noted, are quite resistant and can grow and divide in fairly high concentrations. Inhibition frequently occurs in the range 0.002-0.01 mM. Development of red algal (*Plumaria elegans*) sporelings is reduced 75% by 0.0077 mM arsenite (Boney et al., 1959), the growth of cress, onion, and pea seedlings is inhibited by 0.005 mM arsenite (Cobet, 1919), elongation of oat coleoptiles is depressed 55% by 0.01 mM arsenite (Thimann and Bonner, 1949), *Cecropia* silkworm spermatogenesis is 50% inhibited by 0.002 mM arsenite (Schneiderman et al., 1953), and tissue cultures of chicken fibroblasts are inhibited almost completely by 0.005 mM arsenite (Meier, 1933), of connective tissue 30% by 0.004 mM arsenite (Bucher, 1950), and of rat lymph node 50% by 0.0022 mM arsenite (Trowell, 1960). These data will serve to illustrate the potency of arsenite and it is likely that the organic arsenoxides would be even more effective, as has been shown in the recent study of Savchuck et al. (1960) on cultures of various mammalian cells (see accompanying tabulation). Earle's L-929 strain mouse fibroblast is inhibited minimally by 0.000067 mM phenylarsenoxide and completely by 0.00067 mM. No

<table>
<thead>
<tr>
<th>Arsenical</th>
<th>Concentration for 50% growth inhibition (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Earle's L-929</td>
<td>Skin 2198</td>
</tr>
<tr>
<td>Phenylarsenoxide</td>
<td>0.00039</td>
</tr>
<tr>
<td>Arsenite</td>
<td>0.0025</td>
</tr>
<tr>
<td>p-Nitrophenylarsonate</td>
<td>0.14</td>
</tr>
<tr>
<td>3-Nitro-4-hydroxy-phenylarsonate</td>
<td>0.62</td>
</tr>
<tr>
<td>Atoxyl</td>
<td>0.98</td>
</tr>
</tbody>
</table>
evidence of growth stimulation was observed at any concentration, in contrast to the reports occasionally made that arsenite accelerates mitosis at very low levels.

Mitotic inhibition may arise by different mechanisms and it is first necessary to inquire whether the action of the arsenuclals is primarily on the cytoplasm or on the nucleus. Arsinites and arsonates potently interfere with cell division in fibroblast cultures and the mitotic aberrations are of two general types: (1) a colchicine-like effect on the spindle with metaphase arrest, scattering of the chromosomes in the cytoplasm, and failure in equatorial constriction, and (2) an effect mainly on the chromosomes, manifested by distortion and fusion, separation irregularities (bridges and stickiness), and occasional fragmentation (King and Ludford, 1950). The dialkyl arsenuclal oxide usually produce the former effect, while the pentavalent alkyl and aryl arsonates usually give the latter picture. The concentrations at which they are active are shown in the accompanying tabulation. From

<table>
<thead>
<tr>
<th>Arsenoxides</th>
<th>Arsonic acids</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Me)₂AsOH</td>
<td>MeAsO(OH)₂</td>
</tr>
<tr>
<td>(Et)₂AsOH</td>
<td>n-ButAsO(OH)₂</td>
</tr>
<tr>
<td>(n-But)₂AsOH</td>
<td>ρ-AsO(OH)₂</td>
</tr>
<tr>
<td>ρ₂AsOH</td>
<td>(0.03-0.10 mM)</td>
</tr>
</tbody>
</table>

this work it thus seems that arsenuclals may act differently, this being dependent on their valency state and substituent groups, and that effects may occur on both the cytoplasm and nucleus. It is unfortunate that monosubstituted phenylarsenuclal oxides were not tested for comparative purposes. Fell and Allsopp (1946 a, b) studied the effects of lewisite on tissue cultures and also found both cytoplasmic and nuclear changes. Cell processes contract, surface blebs appear, fat globules fuse into larger droplets, and vacuolization occurs; the nucleus becomes pycnotic and the chromosomes may be clumped into a single mass. The lethal concentration for lewisite oxide is around 0.01-0.02 mM. When the cells are exposed to a sublethal concentration (0.008 mM) of lewisite oxide for 24 hr, recovery occurs on the addition of dimercaprol, indicating that an antimitotic action is not accompanied by irreversible changes. Some differential action was assumed by Haas (1941) in Anodonta eggs, since nuclear damage occurs with 0.15 mM arsenite whereas it requires 0.77 mM for cytoplasmic changes. Brock et al. (1939) also interpreted their results on sea urchin eggs as a primary effect of arsenite on the nucleus, but the evidence for this is not apparent. When Euglena is kept for long periods in 1 mM arsenite, nuclear changes occur, manifested by increased Feulgen staining and coarser granulation.
and attributed to an increase in thymonucleic acid, while simultaneously no cytoplasmic changes could be detected (Rybinsky and Zrykina, 1935). Some cells, such as yeast, do not readily exhibit nuclear damage with the arsenicals (Janke and Garzuly-Janke, 1936), but it is difficult to determine whether this is due to a natural resistance or difficulty in nuclear visualization. Arsenite at 1 mM does not induce the yeast → mycelium conversion, although growth is well inhibited; since mitotic inhibition generally diverts the cells from division to growth without division, this might indicate that mitosis is not specifically blocked and that growth processes are simultaneously affected (Nickerson and Van Rij, 1949).

In most instances, both nuclear and cytoplasmic damage can occur but, in others, at least a partially specific effect on the nucleus may be achieved. From what little we know of the nature of the structural and contractile elements of the mitotic spindle, it certainly seems likely that arsenicals might interfere with its formation or function. It is difficult from cytological studies to localize the site of action, inasmuch as the nuclear-cytoplasmic relationship is such that changes in one can bring about changes in the other. Arsenicals, in common with other antimitotic agents, more readily depress mitosis in rapidly than in slowly proliferating cells, and Florijn (1950) has discussed this in terms of the greater metabolism and energy requirements of proliferating tissues, although earlier work on the metabolism of dividing cells indicates that a qualitative, as well as a quantitative, change in pattern occurs.

A series of reports from Thimann and his collaborators at Harvard has contributed to our understanding of plant growth and the effects of arsenite. Sections of pea stem about 20 mm long, floated on water in the dark for 24 hr, grow in length about 4 mm and with added auxin about 10 mm. Arsenite seems to suppress the stimulation brought about by auxin, although this cannot be definitely shown from the data since the effects of arsenite in the absence of auxin were not observed (see accompanying tabulation)

<table>
<thead>
<tr>
<th>% Elongation</th>
<th>Water</th>
<th>Auxin</th>
<th>Auxin + arsenite</th>
<th>% Change in the auxin response</th>
</tr>
</thead>
<tbody>
<tr>
<td>20.0</td>
<td>50.9</td>
<td>26.5</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>% Weight increase</td>
<td>22.2</td>
<td>58.8</td>
<td>24.0</td>
<td>- 94</td>
</tr>
<tr>
<td>% Glucose decrease</td>
<td>25.9</td>
<td>23.2</td>
<td>36.6</td>
<td>+ 58</td>
</tr>
<tr>
<td>Respiration (2 hr)</td>
<td>5.52</td>
<td>6.36</td>
<td>5.51</td>
<td>-100</td>
</tr>
</tbody>
</table>

(Christiansen et al., 1949). The arsenite concentration used here is 0.1 mM; the growth is somewhat inhibited by 0.01 mM and almost completely prevented by 1 mM (Thimann and Bonner, 1949). The auxin-stimulated
deposition of cell wall material is reduced by 0.1 mM arsenite, as is the respiration, and thus the increased disappearance of glucose is not due to increased oxidation or polysaccharide formation (Christiansen and Thimann, 1950 a). Arsenite inhibits the uptake of water by both potato (Hackett and Thimann, 1950) and artichoke (Hackett and Thimann, 1952) tissue, concentrations in the range 0.01-0.03 mM being 50% effective. What part this plays in the growth inhibition, if any, is unknown, i.e., whether it is a cause or a result. It is important to note that these effects are reasonably specific on growth since the tissues are not damaged over the intervals used. The utilization of endogenous amino acids and the synthesis of protein are readily inhibited by arsenite, but again it is impossible to say whether this is primary or secondary.

**CARCINOSTATIC AND CARCINOGENIC ACTIONS**

Arsenite is now occasionally used in myelocytic leukemia and other blood dyscrasias on the basis of an action which is probably only an extension of its propensity to depress hematopoietic tissues, and perhaps dependent on a selectivity arising from the more rapid proliferation of these cells. Other types of neoplastic cells may be inhibited experimentally. Cutler and Bradford (1878) first reported the treatment of normal, anemic, and leukemic patients with Fowler’s solution. In normals, there was a progressive decrease in erythrocytes and leucocytes; in anemias, a temporary increase was followed by a fall in the erythrocytes; in leukemic patients, the white count fell from nearly 1,000,000 to 9000 while the erythrocytes went from 3,000,000 to 2,000,000. On the basis of this generally ignored report, Forkner et al. (1937) treated ten patients having chronic myeloid leukemia with arsenite and observed a marked decrease in the white count, the spleen also returning toward normal. Since that time arsenite has been one of the effective suppressive agents in such conditions, but recently is being supplanted by possibly more specific drugs. Dustin and his collaborators showed many years ago that cacodylate (dimethylarsinite) causes mitotic irregularities when injected into animals, and later demonstrated the inhibition of sarcoma growth (Dustin and Gregoire, 1933). Ludford (1936) confirmed this but found the sarcoma cells to be only delayed in mitosis and not killed or prevented from resuming growth. Cacodylate at 2.3 mM in vitro slowly produces mitotic inhibition and there is an accumulation of metaphases around 24 hr; higher concentrations arrest mitosis at metaphase in tissue cultures without the formation of an equatorial plate. Sarcoma 37 in mice is damaged by subcutaneous injections of various arsenicals, particularly the trivalent; of 39 trivalents examined, 30 produced some effect (Leiter et al., 1952). Phenylarsenoxide, for example, induced tumor damage in all animals by 24 hr at a dose of 1.5 mg/kg, which is near the
just tolerated dose, and usually a fraction of the animals die, so that the
effect is not specific. Various arsenicals have been tested for the inhibition
of tumors during the past 100 years, usually without remarkable results.
Beck (1953) found trivalent arsenicals to damage mouse lymphomas and
the Harding-Passey melanoma, but again at doses causing toxic effects.
The survival time of mice with Ehrlich ascites tumor is increased some 25%
by the administration of arsenite at 10 mg/kg/day, and the cell count in
the ascites fluid is reduced after 10 days (Creech et al., 1955); repetition of
this work led to a 13% extension of survival (Di Paolo, 1963). Atoxyl
seems to be much more effective, inasmuch as it reduces the ascites mass
from 4.3 to 0.94 ml in 11 days at a dose of 0.2 mg/mouse/day, which is
far below the LD$_{50}$ (Osswald, 1956). Mitotic reduction in ascites smears
was noted, but at this dose there was no chromosomal fragmentation. The
organic arsenicals have not been adequately tested for general carcinostatic
activity. It is interesting that oxophenarsine has a wider range of action
than arsenite in HeLa cell cultures (Toplin, 1959). The ratio of the lethal
end-point concentration (to cause complete degeneration) to the cytotoxic
end-point concentration (to cause some degeneration in 5 days) for arsenite
is around 2.3, whereas for oxophenarsine it is 5.

Whether the carcinostasis is due to an action on the nucleus or to some
metabolic inhibition is not known; however, some results point to the latter.
For example, the cytochrome oxidase of Sarcoma 37 is reduced by oxophenarsine
and can be almost completely eliminated at high doses (Leiter et al., 1953). There was evidence of cellular damage and possibly the loss
of the enzyme is only secondary, especially as cytochrome oxidase itself is
not sensitive to the arsenicals. The respiration of tumor tissue is depressed
by the arsenicals but to no greater degree than that of normal tissues
(Dresel, 1926; Warren, 1943). It seems that the carcinostatic activity, as
far as we know, is not due to enzyme or metabolic inhibition but, inasmuch
as no serious attempts to correlate these actions have been made, one should
suspend judgment until the appropriate data are available. One cannot
avoid the conclusion that arsenicals are often specifically antimitotic,
although we do not understand why, and that carcinostasis is not the result
of a selective action on neoplastic tissue simply because it is neoplastic.
Arsenicals are not taken up to a greater extent by tumors than by corre-
spounding normal tissues. It is true that meningiomas may accumulate
somewhat more arsenite than brain, but this is not a valid comparison, and
carcinomas show no difference (Mealey et al., 1959). Ependymoblastomas
take up less atoxyl than liver or kidney, but more than muscle (Mego and
McQueen, 1963). In all probability the effects are more subtle than could
be observed on the systems studied.

It is definitely established that the prolonged administration of arsen-
ite to man will lead to characteristic and sometimes dangerous changes in
the skin. The first to direct attention to this was Sir Jonathan
Hutchinson in 1887 when he described five cases of skin cancer,
which he attributed to the chronic ingestion of arsenite. By 1930
sufficient cases had been reported so that the condition could
be characterized definitely as an arsenical multiple superficial
epitheliomatosis, which is generally preceded by local
areas of hyperkeratosis. Fowler's solution* taken over a period of years
has generally been the etiological factor. There is a patchy melanosis and
there may be areas of dermatitis, multiple seborrheic warts, and abnor-
malities of the hair and nails; the hyperkeratosis occurs mainly on the palms
and soles, but may be found elsewhere. The cancers are usually epithelio-
as of the squamous cell type and malignant. It has been assumed that arsenic
retained in the skin acts as an irritant to induce neoplastic changes
(Fran-
seen and Taylor, 1934), but actually it is not even certain whether arsenic
is the active agent or indirectly causes the accumulation of some carcino-
genic substances. It is true that the lungs of pulmonary carcinoma patients
contain more arsenic than normal lungs (7.14 and 4.13 μg/g As₂O₃, respect-
ively) (Holland et al., 1960), and for many years attempts have been made
to correlate arsenic intake or tissue content with the incidence of cancer;
but even though such a correlation exists, it implies nothing relative to
mechanism. Experimental topical application of arsenite to the skin has
generally given negative results, although Leitch and Kennaway (1922)
reported that one skin cancer occurred in 100 mice painted with 0.71 mM
potassium arsenite solution for 4 months. Arsenite has been tested in a
variety of ways for carcinogenic activity with generally negative results
(Hartwell, 1951). Injected into bones for several years, arsenite produced
giant cell sarcoma in one case and some muscle tumors when administered
to fowls. Intraperitoneally, it caused peritoneal nodules and one tumor
of the diaphragm. Subcutaneously in the neck, it caused one sarcoma of
the ear. Other instances of tumor induction have been reported, but in all
cases the incidence is very low and there is some question as to the causative
role of arsenite. Since skin tumors are unquestionably related to arsenite
administration, it is not unlikely that other tumors may occasionally be
induced. It is improbable that an inhibition of keto acid oxidation would
be directly carcinogenic, although consequent metabolic disturbances could
conceivably predispose to cancer. The selective suppression of oxidative
processes might, according to certain hypotheses of carcinogenesis, bring
about a pattern of metabolism characteristic of tumor tissue, but whether
such will induce cancerous changes is not known. So little is known of the
effect on nucleic acid metabolism that no conclusions can be drawn as to
this. A good review of arsenical carcinogenesis is given by Neubauer (1947),
and F. Roth (1956, 1957, 1958) has not only reviewed the subject in an

* The daily dose of Fowler's solution is probably around 0.1 ml and thus 100 mg
As₂O₃, or perhaps twice this amount in some cases.
interesting way but has been concerned primarily with the occurrence of skin, lung, and liver tumors in Moselle vintners, who for many years ingested quite large amounts of arsenic in their wines (a mean of 57.3 g over a 12-year period).

**EFFECTS OF pH ON ARSENICAL INHIBITION**

The actions of the arsenicals may be modified by many factors and most of these have been discussed in previous sections, e. g., the effects of enzyme purity, O₂ tension, thyroid, tocopherol, and thiamine deficiency on the respiratory and glycolytic responses, and of temperature. The effects of pH on the inhibition of enzymes by the arsenicals were also summarized (page 649) and thus the only major topic left to be discussed is the pH factor in cellular systems. The modifications of arsenite uptake in *Valonia* and flesh fly larvae by pH have been mentioned previously (page 699).

The inhibition of yeast respiration by 1 mM phenylarsenoxide is modified by pH (see accompanying tabulation) and it was claimed that these results are in harmony with the amphoteric character of this arsenical (Voegtlin *et al.*, 1931). It is difficult to see how phenylarsenoxide could be considered as amphoteric in this range of pH, and equally difficult to understand why the inhibition would increase at pH 7.84, even if an anion is being formed, unless the reaction is at the cell surface and penetration is unnecessary. The inhibition of yeast fermentation by arsenite is not altered between pH 3 and 6, but is moderately reduced above pH 7, which is possibly related to the ionization of arsenious acid (Brady *et al.*, 1961). There is little if any change in the keto acid accumulation in *Microsporum audouini* induced by arsenite over the pH range 5.4–8 (Chattaway *et al.*, 1956). The results on these organisms thus imply that the pH is a rather unimportant factor in arsenical action and this is what would be expected.

A very thorough study of the effects of pH on the trypanocidal action of the acidic phenylarsenoxides was made by Eagle (1945), wherein showing the trypanocidal activity to be strongly dependent on the pH in the manner expected if penetration into the cells involves the un-ionized forms. As the pH is lowered from 8.4 to 5.65, a 10- to 100-fold increase of
the relative molar activity is observed (Fig. 6-10). The activity of nonacidic derivatives (e.g., \( p\)-CONH\(_2\)-\( \eta\)-AsO) shows little or no dependency on the pH over this range, indicating that it is the substituted acidic group which is involved and not the arsenoxide radical. Furthermore, at any one pH, the order of activity is approximately that of increasing \( pK_a \), as expected; e.g., \( p\)-SO\(_3\)H-\( \eta\)-AsO, with a very low \( pK_a \) of 2.0, is never to any extent un-ionized in the pH range studied, and is fairly inactive. Penetration was believed to be the important factor, inasmuch as no differences in reactivity of the arsenicals with thiols can be observed when the pH is altered. Eagle made some ingenious calculations to support this concept. The total activity at pH 8 was assumed to be due to the ionic species, and then the activity to be expected at pH 6 was calculated on this basis and compared with the observed activity at pH 6 (Table 6-17). If the assumptions made are valid, one can estimate the contributions made by either the acid or ionic form at pH 6 for each arsenical. The agreement is satisfactory, considering the various complicating factors, such as preferential binding of the acid form

![Figure 6-10: Effects of pH on the trypanocidal activities of various arsenicals. The substituent groups on phenylarsenoxide and the \( pK_a \)'s are shown to the left of each curve.](From Eagle, 1945.)
### Table 6-17

**Calculated and Observed Trypanocidal Activities of Acidic Arsenicals at pH 6**

<table>
<thead>
<tr>
<th>Phenylarsenoxide</th>
<th>$pK_a$</th>
<th>% Present as:</th>
<th>Activity at pH 8</th>
<th>Calculated activities for:</th>
<th>Observed activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Ion</td>
<td>Acid</td>
<td>Ion at pH 6</td>
<td>Acid at pH 6</td>
</tr>
<tr>
<td>o-SO$_3$H</td>
<td>&lt;2.0</td>
<td>&gt;99.99</td>
<td>&lt;0.01</td>
<td>&lt;0.015</td>
<td>&lt;0.015</td>
</tr>
<tr>
<td>p-SO$_3$H</td>
<td>2.0</td>
<td>99.99</td>
<td>0.01</td>
<td>0.042</td>
<td>0.042</td>
</tr>
<tr>
<td>3-NO$_2$-4-COOH</td>
<td>2.6</td>
<td>99.96</td>
<td>0.04</td>
<td>17.0</td>
<td>17.0</td>
</tr>
<tr>
<td>p-OCH$_2$COOH</td>
<td>3.3</td>
<td>99.8</td>
<td>0.2</td>
<td>0.33</td>
<td>0.33</td>
</tr>
<tr>
<td>p-COOH</td>
<td>4.0</td>
<td>99</td>
<td>1</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>3-NH$_2$-4-COOH</td>
<td>4.7</td>
<td>95</td>
<td>5</td>
<td>0.6</td>
<td>0.57</td>
</tr>
<tr>
<td>p-CH$_2$CH$_2$CH$_3$COOH</td>
<td>4.9</td>
<td>92.7</td>
<td>7.3</td>
<td>6.9</td>
<td>6.4</td>
</tr>
<tr>
<td>p-CH$_2$CH$_2$CH$_2$COOH</td>
<td>5.23</td>
<td>85.4</td>
<td>14.6</td>
<td>3.7</td>
<td>3.2</td>
</tr>
<tr>
<td>3-NH$_2$-4-CH$_2$CH$_2$COOH</td>
<td>5.34</td>
<td>82</td>
<td>18</td>
<td>3.0</td>
<td>2.5</td>
</tr>
<tr>
<td>p-CH$_2$CH$_2$CH$_3$CH$_2$COOH</td>
<td>5.35</td>
<td>81.7</td>
<td>18.3</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td>o-COOH</td>
<td>5.55</td>
<td>74</td>
<td>26</td>
<td>0.52</td>
<td>0.39</td>
</tr>
</tbody>
</table>

---

*From Eagle (1945).*
by the trypanosomes, resulting in a shifting of the equilibrium. Evidently
these different derivatives depend for their activity on factors other than
pH and penetration, and it would be valuable to have data on the pH
dependency of the arsenical inhibition of trypanosomal respiration or
pyruvate oxidation to determine how much this contributes to the devia-
tions from the expected behavior. It should be pointed out that it is not
known to what extent the internal pH changes with the external pH. In
any event, these results emphasize the major importance of penetration
in this group of arsenicals.

**TISSUE DISTRIBUTION**

The distribution of arsenicals between the tissues of animals in certainly
of significance in determining the toxicity pattern. A selection of results is
given in Table 6-18 wherein the distributions of arsenicals can be compared
in several ways.* The four major factors which influence distribution are:
(1) the arsenical used, (2) the species, (3) the route of administration, and
(4) the time after administration. The figures in the table thus represent
cross sections, as it were, of individual instances and are not indicative of
the generalized picture of distribution. We shall discuss these four points
briefly before proceeding to the mechanisms involved and the relations
between distribution and action.

**Dependence of Distribution on the Arsenical**

Even a cursory glance at the table will make it clear that marked differ-
ences between the arsenicals exist. A particularly good comparison may
be made between phenylarsenoxide, phenylarsonate, and oxophenarsine
in rabbits since all were used and determined in the same way. Even oxo-
phenarsine and phenylarsenoxide differ significantly, particularly in such
tissues as the lungs and heart, and there are further differences between
the oxidized and reduced forms, although this latter is obscured somewhat
by the oxidation and reduction which may occur in the tissues (i. e., the
distribution pattern of phenylarsonate may be altered by a fairly large
fraction of it being reduced soon after administration). The studies of
Murgatroyd et al. (1934) comparing the trypanocidal levels in serum after
injections of tryparsamide and tryparsamide oxide suggest that reduction
must occur before activity appears, and that this occurs quite rapidly,

* Radioactive arsenic has been known since 1938 and has been incorporated as
As\(^{74}\) (half-life = 19 days) or As\(^{76}\) (half-life = 26.4 hr) into various compounds in
order to trace the fates of these substances in the body. Such isotopes have been very
valuable for this work, but it is clear that the activity as determined is not necessarily
in the form of the administered arsenical.
the activity being high within 30 min after intravenous injection (Fig. 6-9). The distribution in the tissues would then reflect a combination of both forms. Excretion of the arsenicals is mainly in the pentavalent forms. Injection of phenylarsenoxide or phenylarsonate into rabbits leads to urinary excretion of the latter (Crawford and Levvy, 1947), and administration of arsenite to man results in 70–80% excretion as arsenate (Mealey et al., 1959). These excretion values, however, do not accurately reflect the situation immediately after injection of the valence state of an arsenical in the blood or tissues.

Some very interesting work comparing the tissue binding and the excretion of various arsenicals was reported by Hogan and Eagle (1944), and it was shown that these factors to some extent determine the toxicity. The compounds most readily bound and the least readily excreted are those with high toxicity (see tabulation on page 724). Equimolar amounts of the arsenicals were injected and the amounts bound or excreted were determined after 48 hr. It is probable that the differences observed here are partly due to the differential permeabilities of the tissues to these compounds. The distribution of carbarsone oxide is somewhat different from that of oxophenarsine (Anderson et al., 1947), particularly with respect to tissues such as liver and muscle, and similar variations with the substituent groups may be observed in other studies, indicating perhaps that uptake rather than binding is the critical process.

Differences between Species

One of the most striking differences was reported by Hunter and Kip (1941), who injected radioarsenite into animals for several days and determined the tissue activity. In rats a large amount is always found in the blood, whereas in guinea pigs, rabbits, and man very little occurs in the blood. Another marked difference is that in the rat there is more in the spleen than in the liver or kidneys, whereas in other species the reverse is true (part of this may be due to contamination of the rat spleen by blood). Differences have also been observed with carbarsone oxide: the liver and kidney of rats do not contain particularly high levels, whereas in rabbits the kidney has the greatest amount, and the liver is not far behind (Anderson et al., 1947). The tissue distribution may be secondarily affected by the degree of binding in the blood by proteins or cells; i.e., the more bound in the blood, the less will be available for the other tissues. Some of these species differences in distribution may account in part for the different toxicity patterns observed.

No studies comparing the various routes of administration have been done under controlled conditions, but there is no doubt that the rate and site at which a substance enters the blood stream are usually important factors in determining the distribution in the body and the rate of excretion.
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Arsenite&lt;sup&gt;b&lt;/sup&gt; (mouse)</th>
<th>Arsenite&lt;sup&gt;c&lt;/sup&gt; (rat)</th>
<th>Arsenite&lt;sup&gt;d&lt;/sup&gt; (man)</th>
<th>Arsenite&lt;sup&gt;e&lt;/sup&gt; (man)</th>
<th>q-AsO&lt;sup&gt;f&lt;/sup&gt; (rabbit)</th>
<th>q-AsO(OH)&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;f&lt;/sup&gt; (rabbit)</th>
<th>Oxophenarsine&lt;sup&gt;g&lt;/sup&gt; (rabbit)</th>
<th>Oxophenarsine&lt;sup&gt;g&lt;/sup&gt; (rabbit)</th>
<th>Atoxyh&lt;sup&gt;h&lt;/sup&gt; (mouse)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>0.69</td>
<td>1.34</td>
<td>9.3</td>
<td>12.1</td>
<td>5.46</td>
<td>1.41</td>
<td>1.56</td>
<td>0.10</td>
<td>1.0</td>
</tr>
<tr>
<td>Kidney</td>
<td>3.39</td>
<td>1.12</td>
<td>5.2</td>
<td>36.9</td>
<td>7.19</td>
<td>3.61</td>
<td>3.24</td>
<td>0.47</td>
<td>0.9</td>
</tr>
<tr>
<td>Muscle</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.68</td>
<td>0.51</td>
<td>0.12</td>
<td>0.28</td>
<td>0.11</td>
<td>0.08</td>
</tr>
<tr>
<td>Heart</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4.92</td>
<td>2.06</td>
<td>0.22</td>
<td>0.23</td>
<td>0.12</td>
</tr>
<tr>
<td>Intestine</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>2.00</td>
<td>0.25</td>
<td>1.88</td>
<td>0.22</td>
<td>—</td>
</tr>
<tr>
<td>Brain</td>
<td>0.08</td>
<td>0.13</td>
<td>0.4</td>
<td>0.15</td>
<td>Trace</td>
<td>0</td>
<td>0</td>
<td>0.03</td>
<td>—</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.07</td>
</tr>
<tr>
<td>Lung</td>
<td>0.97</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>12.20</td>
<td>0.83</td>
<td>0.43</td>
<td>0.38</td>
<td>—</td>
</tr>
<tr>
<td>Testis</td>
<td>0.12</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.13</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.45</td>
<td>4.79</td>
<td>2.5</td>
<td>8.35</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Tissue</td>
<td>3.76</td>
<td>0.03</td>
<td>0.64</td>
<td>0.04</td>
<td>7.68</td>
<td>Trace</td>
<td>32.7</td>
<td>0.12</td>
<td>0.2</td>
</tr>
<tr>
<td>----------------------</td>
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<td>------</td>
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<td>------</td>
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<tr>
<td>Pancreas</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salivary gland</td>
<td>0.64</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bile</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.68</td>
<td>Trace</td>
<td></td>
<td>32.7</td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>1.57</td>
<td>15.05</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.12</td>
<td>0.2</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.24</td>
<td>0.86</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocytes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.87</td>
<td>0.56</td>
<td>0.79</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Note that these values are in different units and were determined at different times so they are not comparable.

* Born and Timofeeff-Ressovsky (1941): radioactive arsenite injected subcutaneously at near-lethal dose of 0.05 mg/mouse — values in terms of radioactivity.

* Hunter et al. (1942): injected subcutaneously at 0.546 mg As/kg/day for 4 days — values of As in milligrams per kilogram wet weight.

* Hansen and Moller (1949): means from 40 cases of poisoning (marked variation) — values in milligrams per cent wet weight.

* Mealey et al. (1959): radioactive arsenite (10% arsenate) injected intravenously — values as per cent of dose per kilogram sample normalized to 70 kg body weight; 1 hr after injection.

* Chance et al. (1945): values of As in multiples of what might be expected on the basis of uniform distribution throughout the body from 1 mg/kg injected intravenously; 1 hr after injection.

* Stokinger et al. (1944): oxophenarsine injected intravenously at dose of 6 mg/kg — values of As in milligrams per kilogram wet weight.

* Mego and McQueen (1963): radioactive arsanilate injected intravenously — values of As in moles per mole protein.
Changes in Distribution with Time

Once bound in the tissues, the arsenicals are released rather slowly as one would expect, and several instances of the arsenical remaining after 2–3 weeks, or longer, have been reported (e.g., Magnuson and Raulston, 1941; Lanz et al., 1950). In most of the studies included in Table 6-18, values are also reported for shorter or longer intervals than those given, so that changes in the distribution can be observed. If one plots the tissue concentrations with time, one obtains a variety of curves reaching their maxima at different times and falling at different rates. The initial distribution to some extent depends on the blood flow to the tissues, while the final distribution depends more on the relative permeabilities and binding powers of the various tissues. The original papers should be consulted for these complex results and special attention should be given to the comparative study of four arsenicals by Chance et al. (1945), where marked differences between the arsenicals may be seen at the 1- and 24-hr determinations.

Distribution studies are not as simple as many believe and there are unfortunately complex problems which have not been solved. In much of the work, blood was not thoroughly removed from the tissues before analysis and there is variable contamination from this source, which may be quite high in tissues like the spleen. We often do not know the form of the arsenic in the tissues, since it is determined either chemically or by radioactivity counting. It is not impossible that some of the compounds are metabolized and the arsenic occurs in other combinations, so that in studies of the organic arsenicals particularly, e.g. oxophenarsine, one should not conclude that the findings represent oxophenarsine itself in the tissues. Recent advance in the chemical analysis of the arsenicals may soon enable this problem to be answered.

Excretion of the Arsenicals

Arsenicals are excreted at different rates, depending greatly on the valence state; the pentavalent arsenicals are usually excreted rapidly by the kidney, since they do not bind readily to the tissues, whereas the trivalent arsenicals become rapidly bound and are excreted only as fast as they are released. A fairly high concentration of arsenical in the bile has often been noted and probably accounts for the relatively high fecal levels and in some instances the large fraction excreted in the feces. For example, when oxophenarsine is given intravenously over 4 days to dogs, 26.8% occurs in the urine, 34.5% in the feces, 26.7% in the tissues, and 12.8% is unaccounted for (Magnuson and Raulston, 1941). Monkeys given carbarsone oxide excrete large amounts in the bile and feces (Anderson et al., 1947). Very similar results are seen with dichlorophenarsine in man where, over a 6-day period following a single intravenous injection, urinary excretion is 18.6–20.8% and fecal excretion 21.6–43.4% (Henning and Kampmeier, 1943). In the
study of Chance et al. (1945) around 80% of the total administered arsenical is accounted for by excretion within a week, and in most studies a very tightly bound fraction seems to remain for long periods. Hogan and Eagle (1944) found urinary excretion within a 48-hr period to vary between 7 and 74% of the injected dose with different phenylarsenoxides. Arsenite behaves somewhat differently than the organic arsenicals. It is apt to be excreted fairly rapidly, as in mice where most of the radioactivity is gone within 12 hr (Born and Timofoeff-Ressovsky, 1941), although in man it may take a few days (Hunter et al., 1942). The excretion seems to be almost entirely renal, neither arsenite nor the organic pentavalents being picked up by the liver and excreted in the bile, as are the organic trivalents.

Consideration of Some Special Tissues

Arsenicals injected into the blood stream, or reaching it by other channels, generally leave it rapidly, to be largely excreted if pentavalent or bound in the tissues if trivalent; it was shown over 60 years ago that arsenite at toxic doses leaves the blood stream very rapidly (Morishima, 1900 a). Arsenicals are retained in the blood of the rat more than in other species, and much of it has been shown to be bound to the globin portion of hemoglobin (perhaps 50–60% of that injected) (Hunter and Kip, 1941) so that the erythrocytes must be considered as potential depots for the arsenicals. The high capacity of the erythrocytes for fixing arsenicals was shown by Hogan and Eagle (1944), who incubated rabbit blood and oxophenarsine for various periods. All of the arsenical up to 1.3 mM oxophenarsine is bound, and above this concentration the per cent bound decreases, although the total amount bound increases so that even at 13 mM the erythrocytes are not saturated, despite beginning hemolysis. The rate of uptake by the erythrocytes varies with the arsenical: with phenylarsenoxide, equilibrium is reached within 5 min, whereas with oxophenarsine it requires 2 hr — the total amounts taken up, however, are the same. Arsenite and the pentavalents are not taken up readily by the erythrocytes. When oxophenarsine or dichlorophenarsine is injected intravenously into man, essentially no arsenical can be demonstrated in the blood after 5-15 min (Henning and Kampmeier, 1943). It was calculated by Fink and Wright (1948 a) that the blood concentration of oxophenarsine in patients receiving the drug clinically would be around 0.05 mM, at which concentration they find about 40% to be bound to the erythrocytes. They believed that the binding is at the cell surface, although their evidence is not valid. They showed that, upon washing the cells, 60–90% is removed and a small fraction remains tightly bound.

Arsenicals penetrate poorly into the central nervous system, a fact often of importance in the treatment of infections, although it is believed that greater penetration may be achieved when inflammation is present.
The drug most commonly used for central nervous system syphilis before penicillin was tryparsamide, on the basis that it seemed to penetrate better. An interesting study was made by Hawking et al. (1937) of the trypanocidal activities in the cerebrospinal fluid after the administration of tryparsamide intravenously to patients. The activity is considerable at 14 hr, maximal at 40 hr, and inappreciable by 80 hr; the total arsenic estimated chemically does not parallel this, being maximal at 14 hr but falling rapidly during the next 24 hr. Only a small fraction of the arsenic present in the cerebrospinal fluid is in the active form — around 3% at 14 hr, 18% at 40 hr, and 5% at 60 hr. If trivalent arsenicals are used, much lower cerebrospinal fluid concentrations are achieved as determined by trypanocidal activity. It may be that penetration occurs in the pentavalent form, which must then be reduced for parasiticidal activity. In any event, the relative lack of central nervous system toxic reactions to the arsenicals, when a potent effect might be expected on a metabolic basis, must be in large part due to this failure to penetrate readily. It is also interesting that skeletal muscle does not take up arsenicals readily (Table 6-18) (Beck and Gillespie, 1954). Whether this is due, as with nerve, to the more critical permeability properties of excitable tissue is not known, but the myocardium does not exclude the arsenicals so effectively.

**Intracellular Sites of Binding**

The interesting problem of the location of the arsenicals within the cells following uptake has scarcely been considered. When radioarsenite is injected into guinea pigs 8 times over 4 days, the activity appears in various tissue fractions (see accompanying tabulation) (Lowry et al., 1942).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>As (moles × 10^-7)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acid-soluble fraction</td>
</tr>
<tr>
<td>Liver</td>
<td>3.5</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.7</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.2</td>
</tr>
<tr>
<td>Brain</td>
<td>0.4</td>
</tr>
<tr>
<td>Spleen</td>
<td>&lt;0.3</td>
</tr>
<tr>
<td>Serum</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Thus the bulk of the arsenic is in the protein fraction, except for serum. It would be interesting to know whether any arsenic is truly attached to lipids, or whether the small amounts observed result from slight protein impurities; also whether that found in the acid-soluble fraction might correspond to arsenate taken in through arsenolysis reactions. It was
further shown that the distribution is not uniform among the tissue proteins, but the protein fractions were separated only on the basis of solubility so that accurate characterization is impossible. It was felt that most of the arsenic is bound to protein SH groups.

**METABOLISM OF THE ARSENICALS**

The metabolism of the arsenicals will be considered in three categories: the breakdown of the more complex arsenicals to active forms, the oxidation-reduction reactions, and the reactions whereby the active arsenicals are chemically altered.

**Splitting of Complex Arsenicals**

It is now well known that the arsphenamines are not themselves active but must be split and oxidized to the corresponding arselenoxides. Results such as those of Voegtlin and Smith (1920), who showed that arsphenamines act slowly, are less potent than the arselenoxides, and cleave in solution, and of Rosenthal (1932 a), who showed that arsphenamine after injection into rats can be recovered to the extent of 10–12% in the liver as oxophenarsine, which could account for the therapeutic activity, paved the way for the demonstration by Tatum and Cooper (1934) that oxophenarsine has a better therapeutic index than the arsphenamines and is more clinically effective. Eagle (1939 a) eventually reported that the arphenamines remain inactive under anaerobic conditions but in the presence of oxygen are oxidized to arselenoxides, even in the absence of tissue. The tetraarsenoacetate:

\[
\text{OOC—CH}_2—\text{As}=\text{As—As}=\text{As—CH}_2—\text{COO}^-
\]

has also been shown to be trypanocidal, but only after splitting to acetoarsenoxide and possibly arsenite (Barbour *et al.*, 1925).

**Oxidation-Reduction Reactions**

We have mentioned the early work indicating that the pentavalents must be reduced before parasiticidal activity is exhibited. We shall now discuss in more detail these changes as they occur in the tissues. There is probably sufficient evidence that under the appropriate conditions arsenite can be oxidized to arsenate, and arsenate reduced to arsenite, in tissues or tissue preparations (Binz, 1897). Reduction of pentavalent arsenicals can occur nonenzymically, and thiols such as glutathione (Gordon and Quastel, 1948) may contribute along with other reductants; oxidation of the trivalent forms can also occur nonenzymically, as with Fe^{+++} in
the presence of oxygen (Wieland and Franke, 1928), but more often is probably enzymic. Spitzer (1898) showed that arsenite incubated with liver extract at 37° for 18 hr is oxidized to the extent of 15%, but it is impossible to say how much of this is nonenzymic. Similarly, arsenate incubated with liver pulp or perfused through the liver is reduced to arsenite, part of this probably being due to the glutathione content of the tissue (Thuret, 1939). Blood with added glutathione also reduces arsenate. The activation of tryparsamide has been associated since the work of Levaditi and Yamanouichi (1908) with an unknown substance, sometimes thought to be nonprotein (Yamanouichi, 1910) and sometimes protein (Terry, 1912). Lourie et al. (1935) could not identify the substance responsible for this reduction in blood but showed it to be in the erythrocytes and not to be hemoglobin. It is evident that, in work with either the tri- or pentavalent arsenicals, some consideration must be given to the possibility of these reactions and effects due to the other member of the redox pair.

The clearest demonstration of the enzymic oxidation of arsenite has come from Australia where cattle-dipping fluid was used as a source of bacteria capable of oxidizing arsenite. Several species of bacteria were found to oxidize arsenite at different rates, most requiring about 3 days to oxidize 50% of the arsenite in a 20 mM solution at 25° (Turner, 1954). It was calculated that 77 kcal/mole could be obtained from such an oxidation but there is no evidence that this energy can be utilized by the cells. The enzyme system responsible for this oxidation is adaptive, not making itself evident unless the cells are grown in the presence of arsenite. The organism most commonly investigated was Pseudomonas arsenoxydans-quinque. A number of properties of this system were studied (Turner and Legge, 1954): the time course of the oxidation is linear; the system is substrate-saturated at about 0.9 mM, is well inhibited by cyanide, azide, and carbon monoxide, and can utilize certain dyes as hydrogen acceptors. A soluble cell-free preparation of this arsenite dehydrogenase was prepared (Legge and Turner, 1954) and the effects of different inhibitors studied, with odd results, in that several substances (pyrophosphate, phenylmercuric nitrate, fluoride, iodoacetate, and thiourea) stimulate, while p-mercuribenzoate inhibits rather weakly, and lewisite oxide has no effect. The question of the cytochromes involved was studied (Legge, 1954), and it was concluded that cytochrome c is not a carrier but can be reduced by arsenite in the intact cell. It is possible that the rate of oxidation observed in intact cells is due to the interaction of the dehydrogenase with the oxidase without mediation of a carrier. It would be interesting to know something about the specificity of this enzyme and if it is truly an arsenite dehydrogenase, as well as to determine if such occurs in other organisms or tissues.
Crawford (1947) pointed out that, although it has been assumed that pentavalent arsenicals must be reduced to the trivalent forms before being active, the evidence is all indirect and no one had demonstrated the presence of the oxidized or reduced products of the organic arsenicals in tissues, and no data had been obtained on whether inorganic arsenic can arise from organic arsenicals in the body. Methods for the improved analysis of the various forms were thus developed (page 607). Rabbits were then injected with both valence forms and the excretion pattern determined (see accompanying tabulation) (Crawford and Levvy, 1947). Thus in whichever form it is injected, the major form excreted is the pentavalent. Furthermore, despite the small amount of phenylarsenoxide formed when phenylarsonate is given, it is enough to account for the parasiticidal action. In the case of phenylarsenoxide the rate of oxidation to phenylarsonate determines the rate of excretion and the toxicity of the compound, so that here we have another important factor in addition to the permeability differences. It may be noted that no arsenite or arsenate was found to be formed from either of these compounds.

### Chemical Alteration of the Arsenicals

Although arsenic is retained in the blood for some time following the intravenous injection of oxophenarsine to rats, the duration of the trypanocidal activity is much shorter (Peters and Wright, 1951). There is a progressive divergence between the arsenic content and the trypanocidal activity after 4 hr, the latter decreasing so that it is very low after 12 hr, whereas the arsenic levels actually rise secondarily after the first day. This loss of trypanocidal activity is more rapid than can be accounted for by transfer of the arsenical from the plasma to the erythrocytes. The trypanocidal activity also disappears when oxophenarsine is incubated with blood *in vitro*, but at a slower rate than *in vivo*. These data suggest a fairly rapid inactivation of oxophenarsine in the rat and that the principal site is extravascular. Little is known about the metabolic fate of oxophenarsine or the other organic arsenicals in the tissues.

One of the most interesting aspects of the metabolism of the arsenicals is their methylation by certain molds to form volatile and toxic compounds.
As long ago as 1815 poisoning in Germany had been ascribed to certain wallpapers containing arsenical pigments and many fatalities were reported. A garlic odor was noted under these circumstances and was associated with some toxic volatile compound, but it was not until 1874 that Selmi suggested that molds might be responsible, and not until 1891 that Gosio began his important studies which showed that certain fungi in culture can release toxic substances from arsenite — since called "Gosio gas" — and attempted to determine its nature (Gosio, 1893 a, b). He worked mainly with *Penicillus* (*Scopulariopsis*) *brevicaule* but showed that some species of *Aspergillus* and *Mucor* could also catalyze the reaction. Gosio believed the volatile substance to be some sort of alkyl arsine and Challenger *et al.* (1933) at Leeds finally identified it definitely as trimethylarsine. An excellent review of this subject was written by Challenger (1945).

The mechanism of this methylation by bread molds has been difficult to unravel. The arsenicals were first thought to react with acetate but little evidence for this was obtained; then formaldehyde was considered as a possible donor but the data would not support this either; finally, it was decided that there is a transfer of a methyl group from some donor such as creatine or betaine, reduction of the arsenical occurring simultaneously. This type of methylation is probably not fundamentally different from many transmethylation reactions known to occur, and similar enzyme systems may be involved. Some fungi act on both arsenite and organic arsenicals, some not on arsenite, and very few attack the phenylarsenoxides (Bird *et al.*, 1948). It is interesting that no ethylation occurs, even when the proper donors are added, nor does dealkylation take place with the formation of arsine or inorganic arsenite. *Trichophyton rubrum* has recently been shown to produce arsines from arsenate but not from arsenite (Zussman *et al.*, 1961). We know nothing about whether such methylations can occur in animals. The presence of a garlic-like odor on persons poisoned by arsenic indicates a volatile product, and unknown volatile substances have been detected in animal tissues (Clements *et al.*, 1951).

**THIOARSENITES**

The biochemical and pharmacological properties of the conjugates between arsenicals and thiols will be considered briefly. One would expect the effects of the thioarsenites upon injection into animals or application to isolated systems to depend mainly on the rate and degree to which they are dissociated into the free arsenicals. If an infinitely stable thioarsenite were used, no action should be observed unless the complex itself possesses some activity unrelated to combination with SH groups. Complexes with less and less stability should exert progressively more rapid and greater effects. The stability of the thioarsenite in solution may be of some impor-
tance, but the behavior in contact with biological material cannot necessarily be predicted from the simple rates of hydrolysis (see page 610), inasmuch as the equilibrium may be shifted in the presence of substances which bind the arsenical more tightly than does the thiol to which it was united initially. In biological systems it is presumably the reaction:

\[
\begin{align*}
S-R' & \quad R - As \\
S-C & \quad \leftrightarrow 2 C-SH \\
& \quad S-C
\end{align*}
\]

where C-SH represents cellular SH groups (which may or may not be vicinal for ring formation), that is the most important with respect to both the rate and degree of effect. Certain thioarsenites are trypanocidal, spirochaetidical, or amebicidal, and are therapeutically active (Anderson et al., 1949; Barber, 1929; Cohen et al., 1931; Strangeways, 1937; Yorke et al., 1931 a). The apparent paradox between the activity of these thioarsenites and the ability of thiols to prevent or reverse the effects of the arsenicals is explained on the basis of the amount of thiol present; in the cases of antagonism, it generally requires an excess of the thiol, so that the equilibrium is shifted to the left, whereas in using a thioarsenite the ratio is equimolar. The toxicity of a thioarsenite may well be less than that of the corresponding arsenical, as Labes (1929 b) found with conjugates of arsenite and cysteine, since, first, there is the time factor so that the arsenical may be released slowly and, second, a good deal may be excreted as thioarsenite.

Several investigations of the therapeutic efficacy of various thioarsenites have been made (see review by Eagle and Doak, 1951), and it has been shown that the therapeutic index may be somewhat greater than for the corresponding arsenical. The therapeutic potency is certainly much greater than for the oxidized forms of the corresponding arsenicals (Yorke et al., 1931 a); for example, the effective trypanocidal dose of tryparsamide in mice is 300 mg/kg whereas that for reduced tryparsamide-thioglycolate is only 1.74 mg/kg. The fact that the activity of these thioarsenites can be antagonized by excess thiol indicates that the activity is indeed due to a splitting of the complex. It is not necessary, of course, that the thioarsenites act in exactly the same manner as the corresponding arsenical. The thioarsenites are generally either more or less lipid-soluble than the arsenicals and may be distributed in the body or cells in a different way. The clinical value of dimercaprol lies mainly in its ability to form soluble thioarsenites which diffuse readily from the cells and are readily excreted. Thus the action of a thioarsenite may be greater on a particular tissue if the combination of the arsenical with the thiol allows it to penetrate better, assuming this to be followed by hydrolysis. This may possibly explain the observations of Koppanyi and Sperling (1947) that rabbits receiving certain doses of
arsenite or dimercaprol alone show no toxic effects but, upon administration of both, marked toxic symptoms appear. Peters and Stocken (1947) also reported that oxophenarsine-dimercaprol is around 20 times more toxic to Colpidium than oxophenarsine itself. The following two compounds:

\[
\begin{align*}
\text{Dithiocarboxymethyl derivative of carbarsone oxide (CC. No. 914)} \\
\text{Dithiocarboxyphenyl derivative of carbarsone oxide (CC. No. 1037)}
\end{align*}
\]

were found by Anderson et al. (1949) to be quite effective in amebiasis and to possess certain advantages over carbarsone or its oxide, especially the increased tissue levels in liver and intestine, and a higher therapeutic index. Thioarsenites are now commonly used in African trypanosomiasis, the conjugate of Melarsen oxide and dimercaprol (melarsoprol, Arsolal) being the most noteworthy example (Williamson, 1962). Very little is known about the effects of the thioarsenites on enzymes. Gordon and Quastel (1948) reported that urease in inhibited a good deal more potently by phenylarsenoxide-thioglycolate than by phenylarsenoxide, and in this case it seems that the mechanism of inhibition is not by a reaction with the SH groups. The effects on electron transport systems have been discussed (page 659).

**ARSINE**

Arsine (AsH₃) is a colorless gas (b. p. = −55⁰C) which dissolves in water to the extent of about 20 vol% at ordinary temperatures; a saturated solution is thus around 9 mM. One may calculate the Bunsen absorption coefficient at 37⁰C to be 0.12 and hence about 5.4 mM. The presence of salts decreases the solubility (Jung, 1939); however, physiological concentrations would lower it only 5%. The solubility of the alkyl arsines is, of course, less than that of arsenic. The presence of proteins appreciably increases the solubility. Solutions of hemoglobin and serum proteins absorb more arsenic than do corresponding salt solutions and this is proportional to the protein concentration (Wolff, 1936). Such binding seems to occur only aerobically (Jung, 1939). Thus the bound material may not be arsenic.
Indeed, it is well known that arsine can be fixed in blood in a nonvolatile form and the formation of arsenite was shown over 100 years ago. When rabbit blood is treated with arsine, 47–60% of the arsenic is found to be nondialyzable, and when crystalline horse hemoglobin solutions are so treated, 33–41% is nondialyzable (Graham et al., 1946). It was suggested that the nondialyzable material consists of two fractions, one arsenite and the other unknown. Arsine is quite unstable to light and heat, breaking down into hydrogen and arsenic. The redox potential of the \( \text{AsH}_3: \text{As} \) couple is \( E_0' \) (pH = 7) = \(-1.16 \) v and hence arsine is readily oxidized, a fact which should be considered in biological work. The alkyl arsines are liquids which are also readily oxidized to the corresponding arsine oxides. The structure of arsine is similar to that of ammonia, and infrared data have shown the bond angles to be \( 91^\circ 35' \) and the length of the As—H bonds to be 1.52 Å (Nielsen, 1952).

The relationship between the effects of arsine and of the arsenicals previously discussed is complex. Arsine is one of the most toxic substances and a few bubbles of the gas may be fatal. Concentrations of 0.04–0.05% (about \( 2 \times 10^{-5} \) mole/liter of air) in the inhaled air are toxic to animals in several hours. In cats \( 8 \times 10^{-6} \) mole/liter inhaled leads to toxic symptoms and death in 12–20 hr; the total lethal dose may be around 7–10 mg. The symptoms of poisoning depend on the rate at which it is introduced into the body, and in chronic poisoning a large part of the effects may be due to the arsenite formed from it. The acute effects are nausea, dizziness, weakness, dyspnea, and loss of consciousness; later there may be oliguria, cyanosis, icterus, reduced blood pressure, and variable central disturbances. The chief effects seem to relate to the blood and the central nervous system. Arsine alters the color of the blood and produces certain changes in the hemoglobin, and extensive hemolysis may occur. Many of the symptoms arise from the hypoxemia resulting from the rapid drop in erythrocytes, which may occur within a few hours, as well as the changes in hemoglobin. Furthermore, the renal damage results mainly from the reduction in available oxygen and the debris from hemolyzed erythrocytes.

The mechanism of the hemolysis has been partially elucidated. Hemolysis occurs \textit{in vitro} but requires the presence of oxygen (Naunyn, 1868). Fixation in a nonvolatile form in the blood is rapid and occurs only aerobically (Meissner, 1913; Hughes and LeVvy, 1947), preceding the hemolysis by a brief period (Thauer, 1934). Hemoglobin is the only blood component reacting readily with arsine (Meissner, 1913). There is no doubt that arsine reacts with hemoglobin in the presence of oxygen catalytically to form a product which is hemolytic, either directly or indirectly. The end product seems to be arsenite (Eulenberg, 1865) and numerous workers have detected its presence following exposure to arsine; in fact, the dialyzable fraction after reaction in the blood is arsenite (Graham et al., 1946). Arsenite, how-
ever, is apparently not the hemolytic substance, or at least does not readily produce hemolysis. The acute toxicity of arsine cannot be explained on the basis of the arsenite formed, inasmuch as arsine is much more toxic; the amount of arsenite formed from a lethal dose would be quite small even with complete conversion. There is always the possibility that arsine penetrates where arsenite does not and releases arsenite in regions where even small amounts are toxic, but there is no evidence for this. The changes occurring in hemoglobin are disputed: some have claimed that methemoglobin is formed (Wolff, 1936; Jung, 1939) and some that it is not (Heubner, 1935). Certainly arsine does not produce methemoglobin directly and, in fact, can reduce methemoglobin. There is some evidence that hemoglobin may eventually be degraded to hematin (Wolff, 1936). Various intermediary products of the oxidation of arsine have been believed responsible for the hemolysis: elementary colloidal arsenic (Labes, 1928; Heubner, 1935), diarsine or $\text{H}_2\text{As-AsH}_2$ (Heubner and Wolff, 1936), hydroxyarsine or $\text{AsH}_2\text{OH}$ (Wolff, 1936), and hydrogen peroxide (Jung, 1939). Another suggestion, for which there is little evidence, is that catalase is inhibited in the erythrocytes by some intermediate of arsine metabolism, or that glutathione is reacted (glutathione is antihemolytic) (Jung, 1939). Thus, although arsine is the most powerful hemolytic agent found in industry, we still do not know the mechanism by which it acts.

There is, unfortunately, very little reported concerning the effects of arsine on other tissues. Direct actions on the central nervous system and damage to the liver and kidneys seem likely, but these are complicated by the hypoxemia. It has been shown to act on the isolated frog heart, anaerobically stopping it in diastole and aerobically stopping it in systole, which led to the idea that hydrogen peroxide might be a factor in its action (Richter, 1941). The respiration of liver and kidney slices is depressed by rather high concentrations of arsine (see accompanying slices) (Hughes and Levvy, 1947). The respiration of liver is thus about 5 times more sensitive than that of kidney. Arsenite is somewhat more inhibitory than arsine in the kidney, and less inhibitory in the liver. It had been generally thought

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Arsine (mM)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
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<td>34</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>2.2</td>
<td>71</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.2</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>4.1</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>5.3</td>
<td>43</td>
</tr>
</tbody>
</table>
that essentially all the arsine is taken up by the erythrocytes and later released in the form of arsenite to the other tissues, but Hughes and Levvy showed that other tissues contain arsenite before hemolysis occurs, so that some of the effects may be due to a direct action on the metabolism of the tissues. These effects probably vary with the rate of administration of arsine; if the amount exceeds that which the erythrocytes can bind, some will enter and directly affect the tissues.

It has been shown that various thiols and dithiols can fairly well prevent the hemolysis caused by arsine, as well as the respiratory depression in tissue slices (Kensler et al., 1946). When arsine is 0.19% in the gas phase, kidney slice respiration is inhibited around 60–70%. The inhibition develops slowly, none being observed during the first hour (the values in the tabulation above were obtained during the third hour), which points to the action of some metabolic product of arsine. Dimercaprol has been claimed to be effective in protecting animals from arsine (Kensler et al., 1946), but its clinical use in poisoning has been disappointing (McKinstry and Hickes, 1957). In those cases in which dimercaprol is effective, as in respiratory inhibition in vitro, one must assume that some intermediary product from arsine reacts with the dithiol, perhaps an early oxidation product such as hydroxyarsine, although some of the antagonism may be through an inactivation of the arsenite eventually formed. The mechanism by which arsine directly alters tissue metabolism are undoubtedly complex and it is doubtful if they will be delineated until more extensive study has been made of the products formed from arsine and their effects on various enzymes and metabolic pathways.
CHAPTER 7

COMPARISON OF SH REAGENTS

The SH reagents are generally used to detect and characterize the SH groups of enzymes. A number of problems involved in such studies were discussed in Chapter II-4 and have been considered in connection with the individual inhibitors. A brief comparison of some of these SH reagents with respect to their actions on enzymes will now be presented. Inasmuch as it is difficult to compare the relative potencies of inhibition from the numerous tables given in the preceding chapters, a few results have been collected in Table 7-1 to give a clearer picture of the great variability in the susceptibilities of enzymes to the different types of SH reagent.

Investigations have been included in the table with the following criteria in mind: (1) three or more SH reagents are examined; (2) the concentrations of the inhibitors used should provide an adequate basis for comparison; (3) all of the inhibitors are tested under the same conditions; and (4) the enzymes should be fairly well inhibited so that the likelihood of an SH mechanism is great. It is obvious, however, that one cannot be certain that all the inhibitions recorded are actually due to reactions with enzyme SH groups. In addition to the ordinary inhibition data, a potency factor has been calculated for more ready comparison, since in most studies the concentrations or degrees of inhibition vary a good deal. These potencies should be taken only as rough estimates for comparative purposes. If the inhibition is assumed to be given by a relationship of the type:

\[ i = \frac{(1)}{(1) + rK_i} \]

where \( r \) can represent any factor by which the inhibition deviates from the purely noncompetitive type, one can manipulate this to write:

\[ \text{Potency} = \frac{1}{K_i} = \frac{ri}{(1 - i)(1)} \]

so that the potency values given are really \( 1/K_i \) if the inhibition is non-competitive, or presumably some constant factors of this if there is a com-
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Source</th>
<th>Inhibitor&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Concentration (mM)</th>
<th>% Inhibition</th>
<th>Potency</th>
<th>Reference</th>
</tr>
</thead>
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<td>Acetate kinase</td>
<td><em>E. coli</em></td>
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<td>10000</td>
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<td>0.0003</td>
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<td>3300</td>
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<td></td>
<td>ISBZ</td>
<td>0.3</td>
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<td>3</td>
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<td></td>
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<td>50</td>
<td>1</td>
<td></td>
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<td>Acetyl-CoA carboxylase</td>
<td>Wheat germ</td>
<td>Hg&lt;sup&gt;++&lt;/sup&gt;</td>
<td>0.01</td>
<td>100</td>
<td>&gt;10000</td>
<td>Hatch and Stumpf (1961)</td>
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<tr>
<td></td>
<td></td>
<td>Cd&lt;sup&gt;++&lt;/sup&gt;</td>
<td>0.01</td>
<td>99</td>
<td>9900</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>p-MB</td>
<td>0.01</td>
<td>90</td>
<td>900</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Butarsen</td>
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<td>350</td>
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<td></td>
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<tr>
<td>Aconitase</td>
<td>Pigeon muscle</td>
<td>Hg&lt;sup&gt;++&lt;/sup&gt;</td>
<td>0.05</td>
<td>38</td>
<td>12</td>
<td>Krebs and Eggleston (1944)</td>
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<tr>
<td></td>
<td></td>
<td>Alloxan</td>
<td>10</td>
<td>78</td>
<td>0.35</td>
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<td>12</td>
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<td>Adenosinase</td>
<td><em>Vibrio cholerae</em></td>
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<td>0.07</td>
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<td>&gt;1400</td>
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<td>100</td>
<td>&gt;330</td>
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<td>156</td>
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<td></td>
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<td>Ag&lt;sup&gt;+&lt;/sup&gt;</td>
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<td>Hg²⁺</td>
<td>p-MB</td>
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<td>(Acetobacter suboxydans)</td>
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<td>(Aldolase)</td>
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<td>0.001</td>
<td>0.007</td>
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Comparisons of SH reagents:
- Barron and Levine (1952)
- King and Chekden (1956)
- Nakayama (1961 a)
- Stoppani and Milstein (1957 b)
- Mahler et al. (1954)
- Herbert et al. (1940)
<table>
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<tr>
<th>Enzyme</th>
<th>Source</th>
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<th>Concentration (mM)</th>
<th>% Inhibition</th>
<th>Potency</th>
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<td></td>
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<td>3</td>
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<td><em>Bacillus subtilis</em></td>
<td>Hg²⁺⁺</td>
<td>0.1</td>
<td>16</td>
<td>1.9</td>
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<tr>
<td></td>
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<td>0.09</td>
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<sup>a</sup> Inhibitor abbreviations are:
- IA iodoacetate
- IAM iodoacetamide
- ISBZ o-iodosobenzoate
- p-MB p-mercuribenzoate ion
- p-MPS p-mercuriphenylsulfonate ion
- NEM N-ethylmaleimide
- 1,4-NQ 1,4-naphthoquinone
- PM phenylmercuric ion
- p-Q p-benzoquinone
- p-QH₂ p-hydroquinone
petitive component. These potencies may actually have a physical interpretation if a state approaching equilibrium was achieved in the inhibition, if mutual depletion of enzyme and inhibitors did not appreciably occur, and if secondary inactivation has not contributed significantly to the measured inhibition. Since these conditions are seldom realized, the potencies should be taken only as figures giving very approximate relative inhibitory potencies. They should not be used for comparisons between different enzymes because the experimental conditions are likely to be quite different. If an inhibition is given as 0%, it is assumed that the inhibition is less than 1%, and if it is given as 100%, it is assumed that it is more than 99%, for the purpose of calculating the potencies. Inhibitions given as close to 50% have been chosen when a range of values is given in the reports in order to increase the reliability of the values for comparison.

Only a few general remarks relative to the data in the table will be made and those particularly interested in this type of inhibitor may find some profit in a more detailed perusing of the results. Perhaps it will be only by some such wide comparisons that an understanding of the differences between the various enzyme SH groups will be reached. The fact most immediately apparent from inspecting the table is the tremendous variation in the susceptibilities of single enzymes to different SH reagents, which implies a similar variation in the reactivity or accessibility of SH groups involved in some way in the catalysis. Some enzymes are inhibited strongly by one or more substances but scarcely at all by others, although all these inhibitors are classed as SH reagents. This reflects, of course, the marked differences between the reactivities and other properties of the inhibitors, as well as the differences in the SH groups. Some of the characteristics of enzyme SH groups possibly responsible for their different reactivities were examined in Chapter II-4; these include the electric field surrounding the SH group, steric factors such as would exist if the SH group is buried within the protein structure, the occurrence of disulfide linkages, complexes of the SH group with surrounding groups, and whether there is a single SH group or two vicinal SH groups. One becomes more and more unsatisfied with the common explanation for unreactivity with a particular SH reagent, namely, that the SH group, or groups, is sterically inaccessible, being within some pocket or crevice. First, if the SH group is indeed so situated, then one has the problem of how it is functionally involved in the catalytic process, although of course it is always possible to provide some theory. Second, if the SH group is sterically inaccessible to a certain inhibitor, why does it often react readily with other inhibitors of equal or greater size? It seems that the only valid evidence for a trogloblo- dytic SH group is the demonstration that it does not react with any of the many SH reagents, and even this can be explained in other ways. This problem is only one reason of many why studies on enzyme SH groups
should be made with numerous SH reagents of different types if any degree of characterization is to be attained.

Without doubt the mercurials are generally the most potent inhibitors among the SH reagents. Of the 109 enzymes in the table for which data on the mercurials are available, the mercurials are the most inhibitory in 70%, second most active in 12%, third most active in 9%, and of lower potency in 9%. There are 17 enzymes for which Hg++ and arsenite may be compared, and Hg++ is the more potent in 15, the ratio of potencies being > 1000 in 10; in 25 cases comparing p-MB with arsenite, the mercurial is the more potent in 22, and a ratio of > 1000 is seen in 10. It is difficult to compare the various inhibitors on a quantitative basis, but a rough estimate of the relative activities may be made by averaging the logarithms of the potencies for those enzymes on which pairs of inhibitors have been studied. The logarithm of the potency, as defined above, is really an apparent “pK_i.” Some pairs of inhibitors are collected in the accompanying tabulation; when the designations “mercurials,” “arsenicals,” or “iodo-

<table>
<thead>
<tr>
<th>Inhibitor pairs</th>
<th>Number of enzymes</th>
<th>Mean “pK_i”</th>
<th>Mean potency</th>
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<tr>
<td>Mercurials</td>
<td>52</td>
<td>2.03</td>
<td>107</td>
</tr>
<tr>
<td>Arsenicals</td>
<td></td>
<td>-0.242</td>
<td>0.57</td>
</tr>
<tr>
<td>Mercurials</td>
<td>40</td>
<td>2.26</td>
<td>180</td>
</tr>
<tr>
<td>o-Iodosobenzoate</td>
<td></td>
<td>0.409</td>
<td>2.57</td>
</tr>
<tr>
<td>Mercurials</td>
<td>86</td>
<td>2.065</td>
<td>116</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td></td>
<td>-0.925</td>
<td>0.12</td>
</tr>
<tr>
<td>Arsenicals</td>
<td>43</td>
<td>-0.357</td>
<td>0.44</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td></td>
<td>-0.768</td>
<td>0.22</td>
</tr>
<tr>
<td>o-Iodosobenzoate</td>
<td>26</td>
<td>0.571</td>
<td>3.72</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td></td>
<td>-0.937</td>
<td>0.12</td>
</tr>
<tr>
<td>Hg++</td>
<td>19</td>
<td>2.43</td>
<td>268</td>
</tr>
<tr>
<td>Ag++</td>
<td></td>
<td>2.29</td>
<td>196</td>
</tr>
<tr>
<td>Mercurials</td>
<td>11</td>
<td>2.39</td>
<td>245</td>
</tr>
<tr>
<td>Cd++</td>
<td></td>
<td>1.47</td>
<td>30</td>
</tr>
<tr>
<td>Arsenite</td>
<td>5</td>
<td>0.252</td>
<td>1.8</td>
</tr>
<tr>
<td>Cd++</td>
<td></td>
<td>2.42</td>
<td>263</td>
</tr>
</tbody>
</table>

acetate” are given, it refers to groups rather than individual inhibitor, and in each case the most potent of the group (iodoacetate and iodoacetamide are taken together). These figures show even more clearly the great
relative potency of the mercurials, the only inhibitor having a comparable activity being Ag\(^+\). Ag\(^+\) and Cd\(^{++}\) have been included in Table 7-1 because they react with SH groups (as well as exerting other actions), but will be taken up in another volume with the heavy metal ions. It has been stated that the arsenicals and Cd\(^{++}\) are often specific for vicinal dithiol groups, but, as can be seen in the table, Cd\(^{++}\) is a much more potent inhibitor than arsenite in most cases, although the difference with respect to the uncoupling of oxidative phosphorylation is not so great (Fletcher et al., 1962). It is interesting to compare the arsenicals and the oxidants for the reason that vicinal SH groups might react well with both types of inhibitor. Although the oxidants are around 5 times as potent as the arsenicals on 25 enzymes, there is actually some evidence that a slight correlation between their actions exists. There are, of course, many other factors involved.

The table shows also the relative inactivity of the arsenicals and the iodoacetate-iodoacacetamide group, which is the reason they are not in general useful reagents for the detection or titration of SH groups. However, exactly this lack of a potent action on most enzymes makes them valuable for their selectivity with certain enzymes having the types of SH group with which they readily react.

It is remarkable that some enzymes are inhibited so well by a certain SH reagent and practically not at all by another substance which reacts readily enough with the SH groups of other enzymes. Potency ratios of several million are observed in some instances (e.g., carbonic anhydrase, certain lipases, arginine kinase, \(\beta\)-amylase, ATPase, and aldolase). It should be pointed out that we cannot explain such results at present, although ingenious hypotheses have been suggested. One is left with the conviction that there are factors controlling SH group reactivity which have not been recognized. Perhaps study of the reactions of these different inhibitors with SH-containing simpler substances, such as polypeptides, might help to unravel the mechanisms by which differential inhibitions may be explained.
REFERENCES


REFERENCES

REFERENCES

REFERENCES

REFERENCES


Biochem. Z. 333, 471.


REFERENCES

REFERENCES

J. Biol. Chem. 196, 669.
REFERENCES

REFERENCES


REFERENCES

REFERENCES


REFERENCES


Cochrane, V.V. (1952). *J. Bacteriol.* 63, 459


REFERENCES

Wiley (Interscience), New York.
REFERENCES


REFERENCES

REFERENCES


Dodge, J.D. (1964). *Protoplasma* 58, 312.


REFERENCES


REFERENCES

REFERENCES


REFERENCES

Pharmacol. 209, 235.
York.
Florey, H.W., Chain, E., Heatley, N.G., Jennings, M.A., Sanders, A.G., Abraham,
New York.
Acta 5, 395.
REFERENCES

REFERENCES


REFERENCES

Maruzen, Tokyo.
REFERENCES

REFERENCES

REFERENCES

REFERENCES


REFERENCES

REFERENCES


Hughes, H., Ware, L.L., and Young, F.G. (1944). Lancet i, 148.


REFERENCES

REFERENCES


Joachimoglu, G. (1915). *Biochem. Z.* 70, 144.


REFERENCES

REFERENCES

REFERENCES

REFERENCES

REFERENCES


Laki, K. (1940). Enzymologia 9, 141.
REFERENCES


REFERENCES


REFERENCES


REFERENCES


REFERENCES

REFERENCES

REFERENCES


REFERENCES


REFERENCES

REFERENCES

Ottey, L. and Bernheim, F. (1956). Enzymologia 17, 279.

References

Patterson, W.B. and Stetten, D., Jr. (1949). *Science* 109, 256.


REFERENCES

REFERENCES

REFERENCES

REFERENCES


REFERENCES

REFERENCES

REFERENCES

Van Nostrand, Princeton, New Jersey.
REFERENCES

REFERENCES


REFERENCES


REFERENCES

REFERENCES

REFERENCES


REFERENCES


Wada, Y., Yoshimatsu, H., Koizumi, T., Inoue, F., Ito, K., Morisue, T., Nasu, H.,
REFERENCES

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REFERENCES

Edwards, Ann Arbor, Michigan.
REFERENCES


Yamasaki, T. (1930). Biochem. Z. 228, 123.
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