cial experiment and the ideas of Frisch and Meitner were communicated to a group of physicists, and from which meeting emerged the concept of atomic fission, to be confirmed by experiment in three laboratories within 48 hours.

I fear it would be difficult to recapture the genuine atmosphere, the give and take between earnest men, the tentative hypothesis which collapsed on a sentence, the subtle grasping of relationships which were hardly expressed, the symbolism which crammed into a yard of blackboard the concentrated essence of a generation of mathematics, the mounting tension as revolutionary concepts became clearly formulated and accepted. Something of the sort might be done, and I believe there is many a medical man who, if he participated thoroughly in such an affair, even on a much less ambitious plane, would learn something worth while about how the scientific mind really operates, and what is the method of scientific collaboration at its best.

I suggest, also, that it would help to join a scientist occasionally in serious, responsible discussion of a case, typical or otherwise, of kidney malfunction, or metabolic disorder, or whatever, along with the physician in charge and the staff members. At times the fresh approach, unhampered by tradition and in spite of ignorance, will come up with a clarifying comment under such circumstances. And, in the process, the scientist will grasp more fully the central importance of art in what you do, and the contributive nature of science. He will appreciate the fundamental difference between the analysis of a disease and the forced explicit treatment of a specific case.

Thus there can be a closer approach by each group to the mental processes of the other. But I would go further than this, even though some feel that I may be naive in my approach to a very subtle problem. Men do not learn to understand one another merely by sharing intellectual experiences. They must meet on an emotional level if the foundation is to be built for collaboration on a high plane.

Scientists do not understand the true life of a medical man. With notable exceptions, this is certainly true. Yet all good scientists learn with facility, or else are simply scientists emeriti. Give them a taste of the medical life in its starkest rigor. I remember keenly one of my boyhood experiences, when I accompanied a country doctor through a poverty-stricken hospital. I remember also a conversation with a great friend and an eminent banker, whose maid had been injured by an automobile, and who had just seen the midnight scene in an emergency room for the first time, and whose admiration for the young internes was a joy to witness. I remember also being conducted through a ward, suitably attired so as not to embarrass the patients, with a young surgeon, and watching the devotion in the eyes of a humble woman for whom he had built a new face.

Pick a few outstanding and human scientists and give them such experience, and they will grasp a part of the world of man's experience which they have never known. I do not mean witnessing an operation, where the interest is mainly technical. I mean an introduction to that inner sanctum, where the true heart of medicine throbs strongly, that sanctum which is securely guarded against the cynicism of selfish men, and against the ribald comments of those to whom nothing is sacred. Indoctrinate well and test, communicate the password, and guide. From true scientists the response, while silent, will be all that you hope.

Now, what about this queer notion on the part of scientists, that medical men tend to try to dominate any small group brought together for collaboration. Here I do not know enough about the medical profession to prescribe, although I know quite a lot about some medical men. The ones I have become well acquainted with are entirely free of the fault. Perhaps there is no basis for the rumor. So I will have to leave the treatment of this ill, if indeed the symptoms are real and have not been misinterpreted, to medical men. It may call for properly proportioned psychiatric treatment; I am sure it is no case for surgery. It may be that it merely needs to be given a name and relegated to the category of rare diseases for which there is no cure but which are not of great social moment. Medical men will know. I merely mention that I have heard the allegation.

There are other ways, worth-while no doubt, in which the professions may be brought to a better understanding of one another. It is not necessary that they be brought to a full understanding of one another's subject matter; that would be impossible. For, if they grasp one another's mores and traditions, methods of thought, deep convictions, and motivations, there will be no further need to stimulate collaboration of the highest sort. It will occur automatically. And from it will result a surge forward on that complex task of understanding life, where the skill of all professional groups will be strained to the utmost, a new accomplishment which will place a firmer foundation under the keystone of that honorable profession to which medical men belong, ministry to the people. May that ministry always be conducted with pride and dignity. And may the gratitude of humble men always remain the primary compensation and reward.

# Chemical Aspects of Enzyme Inhibition

Irwin W. Sizer

The subject of enzyme inhibition has come to the forefront in recent years because it offers the chemist the opportunity to study the nature of the active site and the mechanism of enzyme action. To the physiologist, it affords a technique for studying the functioning and coupling of enzyme systems within living cells and tissues. In its application to insecticides, herbicides, antimicrobial agents, and drugs, the concept of enzyme inhibition has not only proved fruitful but has provided a rationale for future developments in these areas. Although this article deals primarily with the chemical aspects of inhibition, major applications to medicine are discussed in which the biological effects of chemical compounds can be interpreted in terms of inhibition of particular enzyme systems.

Ever since the discovery of enzymes, it has been known that they are highly labile molecules which can be readily poisoned by a variety of agents. Modern developments date from 1928, when Quastel and Wooldridge (*1*) demonstrated the inhibition of succinic dehydrogenase by malonate and its reversal by excess succinate. The phenomenon of competitive inhibition was almost for-

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gotten until Woods (*2*) showed that the bacteriostatic action of sulfanilamide could be reversed by its structural analog, *p*-aminobenzoic acid. He proposed the concept that this vitamin was a necessary component of an essential enzyme system which could be displaced by the sulfa drugs. Since that time, the field of antivitamins, antimetabolites, and antienzymes has developed rapidly, and the mechanism of enzyme inhibition has become much better understood.

The foundation of our knowledge of the mechanism of enzyme inhibition was laid by Michaelis and Menten in 1913 (*3*). They proposed that in an enzyme-catalyzed reaction the enzyme first combines chemically with its substrate to form an enzyme-substrate compound (ES), which in turn decomposes to release enzyme end products. In much the same manner, an inhibitor can combine with an enzyme to form an inactive enzyme-inhibitor compound (EI). In the case of catalase (Fig. 1) and certain other enzymes, the reversible formation of ES and EI can be demonstrated by changes in absorption in appropriate regions of the spectrum. Although enzyme inhibition may be reversible upon removal of the inhibitor, numerous examples of partial or no reactivation can be cited.

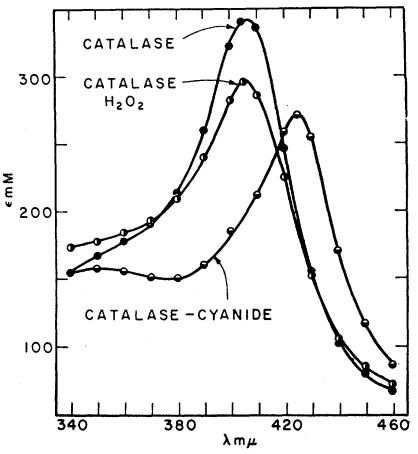



Fig. 1. Absorption in the violet region of the spectrum of catalase, catalase-peroxide compound (ES), and catalase-cyanide (EI) compound (*34*).

## Mechanism of Inhibition in vitro

Although many enzymes are simple proteins, most require the presence of a coenzyme or metal, or both, for activity. An inhibitor might interfere with the catalyzed reaction in several ways (see Fig. 2): (i) by complexing with the substrate and thereby eliminating it; (ii) by combining with the protein-active groups (those that combine with substrate); (iii) by uniting with the catalytically inert part of the protein; (iv) by combining with a cofactor such as coenzyme or metal; (v) by reacting with the enzyme-substrate compound; and (vi) by a combination of the afore-mentioned ways.

In an interpretation of how an inhibitor may act on an enzyme system, an examination of the kinetics of the catalyzed reaction in the presence of inhibitor is invaluable. Variables such as *p*H, temperature, and inhibitor concentration may provide important clues, but most important is a study of the effects of substrate concentration on the inhibited system (Fig. 3).

*Competitive inhibition.* Enzymes are not completely specific, and they activate a family of related compounds. If the relationship is fairly close but not intimate enough for the compound to undergo reaction, then such a substance may act as a competitive inhibitor by combining reversibly with the active site of the enzyme. In a similar way, an inhibitor that bears a structural resemblance to the coenzyme may displace it from the protein surface and thereby interfere with catalysis. Since an inhibitor of this type competes directly with the substrate for the active groups of the enzyme, the ratio of substrate to inhibitor is the critical factor determining the extent of inhibition. When this ratio is high (see Fig. 3), the rate of the inhibited reaction approaches that of the uninhibited one.

In a system involving competitive inhibition, the relationship between the enzymic fractional activity $\alpha$, the inhibitor concentration $I$, and the substrate concentration $S$ is

$$I \cdot \frac{\alpha}{1-\alpha} = K_I + \frac{K_I}{K_S} \cdot S$$

where $K_S$ is the dissociation constant of the enzyme-substrate compound (ES) and $K_I$ is the dissociation constant of the enzyme-inhibitor compound (EI). A plot of this relationship (Fig. 4) enables one to measure directly the dissociation constants of ES and EI and also makes it possible to distinguish between competitive and other types of inhibition.

*Noncompetitive inhibition.* A noncompetitive inhibitor combines with the enzyme at a site other than the active one. Hence, the affinity of the enzyme for substrate is not altered, and no amount of substrate will reverse this type of inhibition (see Figs. 3 and 4). Since noncompetitive inhibition is independent of substrate concentration, the dissociation constant $K_I$ of the enzyme-inhibitor complex is given by the equation

$$I \cdot \frac{\alpha}{1-\alpha} = K_I$$

From a plot of experimental data such as that presented in Fig. 4, it is possible not only to distinguish between competitive and noncompetitive inhibition, but also to calculate in both cases the dissociation constants of enzyme-inhibitor compounds. Because it is not possible to predict the type of inhibition characteristic of a particular enzyme-inhibitor system, a careful investigation will usually require the measurement of inhibition at several inhibitor and substrate concentrations. In view of the fact that a noncompetitive inhibitor may induce extensive changes in the enzyme molecule, this type of inhibition is often irreversible.

*Uncompetitive and other types of inhibition.* In uncompetitive inhibition, the inhibitor combines not with the enzyme but with the enzyme-substrate compound to form an IES complex. As is the case of competitive inhibition, the apparent dissociation constant of ES is decreased, but, unlike the situation characteristic of competitive inhibition, the maximum velocity at high substrate concentration is also decreased (see Fig. 5). Although uncompetitive inhibition is not commonly encountered, it, nevertheless, is important for certain enzyme-inhibitor systems.

A given inhibitor may be simultaneously effective by different mechanisms. For example, it might combine with a free active site, or a site that is already occupied by substrate, or with a group removed from or adjacent to the active site of the enzyme molecule (*4*). In addition, inhibitors may act in nonspecific ways by bringing about irreversible inactivation by protein denaturation, hydrolysis, or oxidation. Changes in *p*H may inhibit enzyme reactions by their effects on the substrate or by altering the ionization of the active groups of the enzyme. Furthermore, *p*H changes may modify the effects of an inhibitor on an enzyme (*4*).

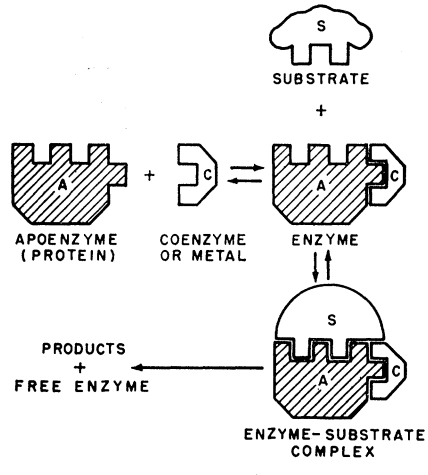



Fig. 2. Schematic diagram illustrating the pathway of many enzyme-catalyzed reactions. For some enzymes, the step involving the combination with coenzymes or activating metal is not required (modified from McElroy, *35*).

## Inhibition Analysis of the Enzymatically Active Site

The architecture, composition, and properties of the groups on the enzyme molecule primarily responsive for catalysis have been elucidated by the use of inhibitors. Unfortunately, many of the group reagents are not completely specific; hence, the most reliable conclusions on the role of a particular group in the enzyme are based on inhibition studies with more than one reagent. The use of such compounds has made it possible to distinguish between the active site and that part of the protein which plays a passive role in catalysis as a carrier of the prosthetic group.

*Primary amino groups.* The significance of amino groups in determining enzyme activity has been investigated by inhibition studies employing reagents for amino groups of proteins. Unfortunately, most reagents are not completely specific and react with other groups such as sulfhydryl and phenol. Most commonly employed reagents for amino groups include ketene, acetic anhydride, phenyl isocyanate, nitrous acid, dinitrofluorobenzene, and formaldehyde. The roles of the amino groups of lysine vary strikingly; these groups are not essential for enzyme activity of pepsin, β-amylase, trypsin, and chymotrypsin, but they are required for α-amylase and alkaline phosphatase (*5–7*).

*Carboxyl groups.* Information on the role of carboxyl groups of aspartic and glutamic acids in the active site is fragmentary because of the difficulty of chemical reactions with carboxyl groups under mild conditions. Esterification with methyl alcohol or 1,2-epoxides suggests the importance of carboxyl groups in ovomucoid and crotoxin. On the other hand, the C terminal groups are not essential for the enzymatic activity of chymotrypsin (*7, 8*).

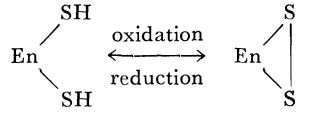
*Phenolic groups.* Acylating agents and nitrous acid react more rapidly with amino than with phenolic groups. Iodine reacts primarily with phenolic groups but also with imidazole and indole. In certain enzymes in which tyrosyl groups are exposed, they may be oxidized by tyrosinase, which is specific for phenolic groups (*9*). By the use of different reagents, the conclusion has been reached that free phenolic groups are required for activity of β-amylase, pepsin, chymotrypsin, phosphatase, thrombin, and invertase, but not required for trypsin and certain other enzymes (*6, 7, 9*).

*Indole groups.* The lack of inhibitors specific for indole groups of proteins renders difficult the assessment of the role of tryptophan in enzymes. Under certain conditions, iodine and formaldehyde react with indole groups of proteins. Peroxidase is capable of inactivating such enzymes as chymotrypsin, invertase, and β-amylase (*9, 10*), and this inhibition appears to be the result of the oxidation of indole groups.

*Imidazole.* The extensive investigations of the mechanism of action of nerve gases including tetra-alkyl pyrophosphates, dialkyl *p*-nitrophenyl phosphates, and dialkyl fluorophosphates have revealed that these compounds are powerful irreversible inhibitors of esterases, including proteases. It appears that these inhibitors phosphorylate a basic group in the esteratic site of the enzyme (*11, 12*). At first it was suggested that serine residues were involved in the reaction with nerve gases, but more recent work implicates imidazole groups. The inhibition of certain enzymes by mustard gas, nitrogen mustards, and diazonium compounds may be due to their reaction with imidazole as well as with other groups.

*Sulfhydryl-disulfide.* Of all the groups on the enzyme molecule, the sulfur of cystine or cysteine has been most extensively studied and appears to be of utmost importance in enzyme-catalyzed reactions. Perhaps one reason for the excellent information available is the wide choice of inhibitors. Metal ions (for example, silver and copper) accelerate the oxidation of sulfhydryl groups, as do oxidizing agents such as ferricyanide, porphyrindin, iodine, mustard gas, *o*-iodosobenzoate, unsaturated ketones, and quinones. Alkylating agents such as iodoacetamide and methyl bromide, and mercaptide-forming compounds including organic arsenicals and *p*-chloromercuribenzoate are useful agents for sulfhydryl groups (*13*).

Results with these reagents indicate that sulfhydyryl groups are essential for the activity of a large fraction of the known enzymes; when sulfhydryl groups are oxidized to disulfide or alkylated, the enzymes are reversibly inactivated:




Active enzyme — Inactive enzyme

Reducing agents such as cysteine, thioglycollic acid, ascorbic acid, hydrogen sulfide, glutathione, and 1,2-dimercaptopropanol (British antilewisite, BAL) are effective in restoring activity. The case of glyceraldehyde-3-phosphate dehydrogenase deserves special mention because the essential SH group belongs to glutathione, which is firmly bound to the protein (*14*). The sensitivity of SH enzymes to oxidation-reduction potential suggests a physiological means of controlling the activity of these enzymes inside living organisms.

It might be surmised that certain proteins would require the disulfide bond for activity, but few examples can be cited other than the well-known one of the hormone insulin. Although urease is a typical SH enzyme, extensive reduction of disulfide groups required for the maintenance of protein structure may result in inactivation (*15*). Clostridial collagenase apparently requires the SS bond and is inactivated by reducing agents (*16*).

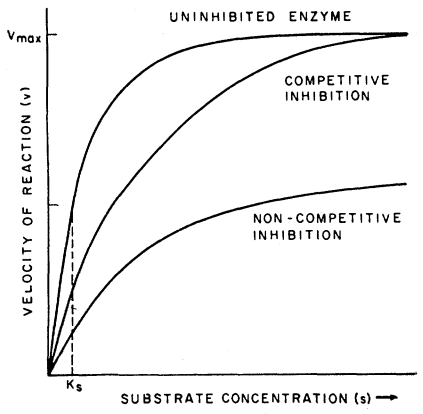



Fig. 3. The rate of the catalyzed reaction as a function of substrate concentration is plotted for an active enzyme and for an enzyme inhibited by either a competitive or a noncompetitive inhibitor (*34*).

*Polypeptide chain.* By no means all of the protein molecule is required for enzyme activity, and in many cases it has been possible to split the polypeptide chain without loss of enzyme activity. Mild conditions of hydrolysis must be used, however, and one is limited primarily to enzymes that split the peptide bond. Proteolytic enzymes as well as amino- and carboxy-peptidases have furnished evidence that in many cases amino acids and peptides can be successively removed from the polypeptide chain of an enzyme before loss in activity occurs. For example, carboxypeptidase can remove 20 percent of the nitrogen of ribonuclease while more than 70 percent of the enzyme activity is retained (see *14*). Although glyceraldehyde-3-phosphate dehydrogenase is inactivated by proteolytic enzymes, its activity is restored by SH compounds (*14*). Pepsin can be partially self-hydrolyzed and retain activity.

*Phosphate.* Certain proteins contain phosphate (attached to the hydroxyl group of serine) that can be readily removed (*17*). In the case of phosphorylase, the removal of phosphate by phosphatase results in complete loss of activity. This inactivation can be reversed by dephosphophosphorylase kinase and adenosine triphosphate. Glucagon and epinephrine may play an indirect role in this reactivation (*18*).

*Metals.* Metals may be firmly bound in stoichiometric proportions to proteins (metalloenzymes) or loosely and nonspecifically bound to proteins (metal-enzyme complexes) (*19*). Included in

the first category are copper, iron, molybdenum, and zinc, all of which are located in the active site of most oxidative (also hydrolytic in the case of zinc) enzymes. Agents with high affinity for the metal and suitable steric configuration completely inhibit the enzyme by formation of a metal-inhibitor complex. Inhibitors that have been especially useful in elucidating the structure of metalloenzymes are cyanide, azide, carbon monoxide, hydrogen sulfide, phenylthiourea, *o*-phenanthroline, 8-hydroxyquinoline, and ethylenediamine tetraacetic acid. If the inhibitor has not separated the metal from the protein, the enzyme can often be reactivated by suitable removal of the inhibitor (*19*).

Loosely bound metal ions that activate enzymes by forming metal-enzyme complexes include magnesium, manganese, potassium, calcium, iron, and cobalt. Metal ions that activate enzymes can be readily removed from the system by dialysis, by chelating agents such as citrate, oxalate, pyrophosphate, and Versene, or by reagents specific for a particular metal. Fluoride is especially useful as an inhibitor of magnesium-activated enzymes such as enolase. Competition among metal ions may occur for the reactive groups of the enzyme; for example, cobalt, copper, and zinc may inhibit the activation of prolidase by manganese; silver and mercury inhibit the activation of arginase by cobalt and manganese; and sodium may interfere with potassium in its activation of glycolysis. The problem of metal ions is complicated by the fact that these cations may also complex with the substrate, thereby facilitating or inhibiting enzyme action (*19*).

*Coenzymes.* Enzyme systems that require a coenzyme may be inhibited by reagents which combine with the coenzyme either before or after its removal from the protein apoenzyme. Especially illuminating have been studies on the substitution for the coenzyme of a compound (anticoenzyme) that resembles the structure of the coenzyme sufficiently closely so that the anticoenzyme displaces the true coenzyme from the apoenzyme. Since the essential group of the coenzyme is usually one of the B vitamins, most of the anticoenzymes fall into the category of antivitamins. Not only can the antivitamin act as a competitive inhibitor in an enzyme system requiring a coenzyme, but in the living cell it may also block the synthesis of the coenzyme itself, causing the organism to develop symptoms of a vitamin deficiency (*20*).

Coenzymes that can be blocked by antivitamins include the pyridine nucleotides, flavin nucleotides, thiamin pyrophosphate, pyridoxal phosphate, coenzyme A, *d*-lipoic acid (6,8-dithiooctanoic acid), and many others. Specificity among the coenzymes is extreme, for example *l*-lipoic acid inhibits the functioning of the coenzyme as do also the analogous 4,8- and 5,8-dithiol acids. Similarly in the case of the pyridine nucleotides, reduction involves the introduction of hydrogen into either the cis or the trans configuration of the para position of the nicotinamide (*21*). From these studies it is clear that only minor alterations in a vitamin molecule may convert it into an antivitamin capable of blocking directly or indirectly the functioning of a coenzyme in an enzyme system. Enzymes containing both coenzyme and metal can be inhibited by reagents for the metal as well as by anticoenzymes.

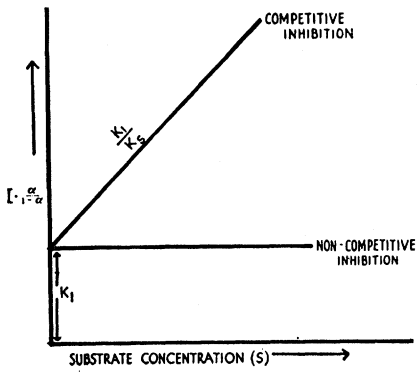



Fig. 4. Inhibitor concentration multiplied by the ratio of inhibited enzyme to active enzyme is plotted as a function of substrate concentration for both competitive and noncompetitive inhibition (*34*).

## Architecture of the Active Site

While previously discussed inhibitors of selected groups have contributed greatly to knowledge of the active site, more direct information has come from studies specifically directed toward that part of the enzyme molecule directly concerned in catalysis. Competitive inhibitors that bear a direct or indirect relationship to the natural substrate have been especially useful. Since such inhibitors interfere with the metabolism of normally occurring compounds, they are called antimetabolites. Also included in this category are the antivitamins, which have already been discussed. Although the antimetabolites are invaluable in mapping out the topography and chemical nature of the reactive group, their contributions to our knowledge of intermediary metabolism, including the citric acid and the urea cycles and biosynthesis in general, are equally significant. Applications to applied problems, especially in agriculture, medicine, pharmacology, dentistry, and nutrition, are developing rapidly and promise exciting vistas for the future.

Use of homologous series of compounds has furnished information on the dimensions of the active site. The introduction of one $CH_2$ group into an inhibitor may greatly alter its action, suggesting that steric configuration of the active site is highly specific. Increase in size by only a few angstrom units may convert an inhibitor into a molecule that no longer has access to the enzymatic site. This is well illustrated by the alcohol dehydrogenase system. The length of the aliphatic chain determines whether the alcohol is a good or poor substrate, an inhibitor, or without effect on the enzyme (*22*).

One of the best examples of inhibition analysis by the use of antimetabolites is given by studies of Nachmansohn, Wilson, and their collaborators (*11, 23*) on acetylcholinesterase. This enzyme is strongly and irreversibly inhibited by certain chemical warfare agents and insecticides belonging to the group of phosphate esters, including tetra-alkyl pyrophosphates, dialkyl *p*-nitrophenyl phosphates, and dialkyl fluorophosphates. The lethal action of these nerve gases is due to an inhibition of acetylcholinesterase which is not reversed by dialysis. These inhibitors phosphorylate a basic group (imidazole ?) of the active esteratic subsite which is normally bound to the ester function of acetylcholine and is involved in the catalytic hydrolysis of the ester linkage. Similar inhibitor studies using antimetabolite analogs of acetylcholine have shown that the active site of the esterase consists of a second subsite, called the anionic site, capable of binding and orienting, by Coulombic and van der Waals' forces, ammonium structures and responsible for binding the choline part of the acetylcholine (see Fig. 6). In addition to inhibition by choline analogs, the esterase is inhibited reversibly at lev-

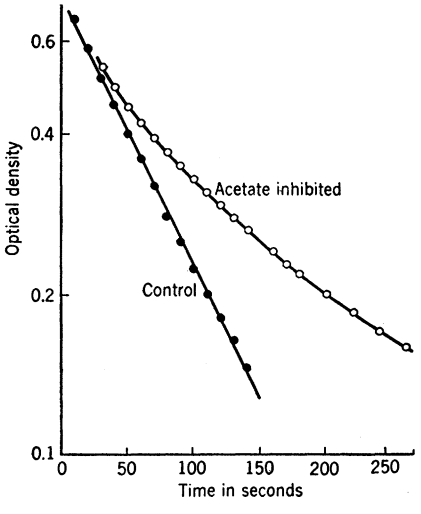



Fig. 5. Catalysis of $H_2O_2$ decomposition by beef-liver catalase in the presence and absence of 0.3*M* sodium acetate in 0.1*M* phosphate buffer, *p*H 6.0 at 25°C; $10^{-2}$ to $10^{-3}$*M* $H_2O_2$ (*36*).

els of $10^{-6}M$ by such powerful alkaloid drugs as eserine, prostigmine, atropine, and curare (*23*).

In the case of the alkyl phosphate-enzyme complex, the inhibition is ordinarily irreversible, since reaction with water to regenerate unbound active enzyme is extremely slow. Some reactivation was obtained with nucleophilic reagents such as hydroxyamine and pyridine, which compete with the dialkylphosphoryl group for the positively charged esteratic site. Far more effective in reactivation were compounds that in addition possessed a cationic center for Coulombic binding to the anionic site. Included in this group of reactivators are nicotinohydroxamic acid and 2-pyridine aldoxime methiodide. The latter compound has been used successfully as an antidote to nerve-gas poisoning and is capable of repairing the chemical lesion produced by the alkyl pyrophosphate (*24*).

## Inhibition in Living Systems

Enzyme inhibitors have profound effects on living organisms by blocking vital reactions of intermediary metabolism. In this way, they may modify the behavior of the cell or organism, affect growth, development, and reproduction, and produce necrosis and death. Interpretation of inhibition is fraught with difficulties because it does not necessarily follow that the locus of action is a particular enzyme system from the fact that an inhibition *in vitro* can be demonstrated. It must be proved that the enzyme under indictment is actually inhibited in the living system at the concentration of inhibitor that is known to have accumulated inside the cell. In addition, the enzyme block must quantitatively explain the biological effect of the inhibitor (*25*).

An example of the difficulties involved is presented by the nitrogen mustards, which have been widely used with partial success in the treatment of certain types of cancer. These cytotoxic materials inactivate hexokinase, adenosine triphosphatase, choline oxidase, esterase, and acetylase, and they interfere with biosynthesis. The explanation of the mode of action of nitrogen mustards is not this obvious, however, for the concentration of the drug required for enzyme inhibition is greater than that found in tissues after a lethal dose has been administered (*26*).

Except in the case of chemical warfare, the biochemist would like to use an enzyme inhibitor like a surgeon's scalpel to differentiate at the molecular level between closely related living systems. In theory, the difference between them may be subtle indeed and need involve only

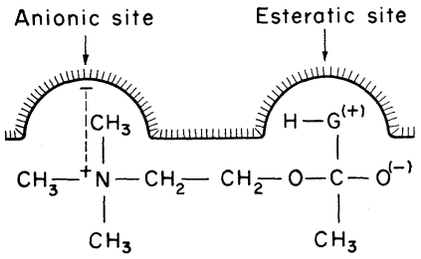



Fig. 6. Schematic presentation of interaction between the active groups of acetylcholinesterase and its substrate (*23*).

one enzyme in a thousand or more; in such a situation, a specific inhibitor might be able to eliminate one of a pair of cells or organisms. While such differential and specific effects of inhibitors do indeed depend on quantitative or qualitative differences in enzymes associated with alternate pathways of metabolism that exist between pathological and normal tissues or between host and parasite, other properties may be equally important. In particular, permeability differences involving cell wall, plasma, nuclear membranes, and mitochondrial membranes (both external and in the christae mitochondriales) are of critical importance in explaining the difference in susceptibility to such biologically active compounds as drugs, antibiotics, and chemotherapeutic agents.

*Inhibition analysis of cell chemistry.* The first use of inhibitors in living systems was as blocking agents in unraveling the successive steps in glycolysis, in the citric acid and urea cycles, and in numerous systems of biosynthesis. A recent addition to the list, which includes such famous inhibitors as iodoacetate, fluoride, and malonate, is fluoroacetic acid, a poison from certain South African plants. In man a "lethal synthesis" of fluorocitrate from fluoroacetate occurs which blocks the citric acid cycle by an inhibition of aconitase (*27*). Inhibition analysis was not only instrumental in elucidating metabolic pathways, but these investigations in turn have validated the theory of enzyme inhibition as applied to living matter.

*Antimicrobial enzyme inhibitors.* Studies on the use of inhibitors as bactericides and fungicides have proceeded at a rapid pace ever since sulfanilamide proved to be a competitive inhibitor that blocks the utilization of *p*-aminobenzoic acid in the synthesis of the folic acid enzymes. Gould *et al.* (*28*) found that bisphenolic compounds such as hexachlorophene strongly inhibit succinoxidase, cytochrome oxidase, and lactic dehydrogenase, but it is not clear that this accounts completely for the bactericidal action of hexachlorophene. Penicillin blocks both protein and nucleic acid metabolism by inhibition of peptide bond synthesis, oxidation of nucleotides, and their formation from purines (*25*). Streptomycin is a nonspecific inhibitor with a marked effect on diamineoxidase. It also interferes with the oxidation of pyruvate and oxalacetate.

Bacteria are more susceptible than mammals, apparently because of the relative impermeability of plasma membrane and mitochondria of the animal cell to streptomycin (see *25*). Chloramphenicol is structurally related to phenylalanine and may inhibit its incorporation into proteins. Its antibiotic properties might be related to its ability to block protein synthesis in bacteria. Antimycin is a powerful inhibitor of succinoxidase. Tetracycline uncouples respiration from aerobic phosphorylation (*29*). This antibiotic action may be prevented by magnesium, which binds the tetracycline. These few examples were chosen to indicate the usefulness of the hypothesis that antimicrobial agents can act as enzyme inhibitors.

*Drugs as enzyme inhibitors.* The concept that the pharmacological action of drugs may be the result of their inhibition of enzyme systems has proved fruitful and now affords a rationale in the search for chemicals with physiological action. Drugs previously discussed, which inhibit acetylcholinesterase, constitute one of the best documented examples in this category. Acetazoleamide (Diamox), which is useful in the treatment of edema, glycoma, and epilepsy, reversibly inhibits carbonic anhydrase even at concentrations of $10^{-8}M$. By interfering with this enzyme in mammals, Diamox prevents a tissue (kidney, stomach, brain, pancreas, and ciliary bodies) from secreting acid or base (*25*). Disulfiram (Antabuse), which is useful in the treatment of alcoholism, apparently inhibits enzymes that oxidize acetaldehyde. Hence disulfiram causes an accumulation of toxic aldehyde after alcohol is administered. This drug has been shown to inhibit copper and SH enzymes (*25*).

Anesthetics and hypnotics produce a decrease in nerve activity accompanied by a slight decrease or increase in oxidation and an interference with the utilization of adenosinetriphosphate. These drugs appear to affect most markedly the stimulated respiration and synthesis of phosphate bond energy of active as contrasted with resting nervous tissue (*30*). In the respiratory chain of the cell, enzymes operating between the pyridine nucleotides and cytochrome *b* constitute the links most susceptible to inhibition by hypnotics and narcotics. Although an encouraging beginning has been made on the mode of action of drugs, much research remains to be done before their action can be explained in terms of enzyme inhibition.

*Inhibitors in cancer chemotherapy.*

The search for inhibitors that distinguish between enzymes of normal and neoplastic cells has not been easy in view of the general similarity of the metabolism of the two types. Most effective in the treatment of cancer have been antimetabolites that interfere with either folic acid enzymes or enzymes involved in nucleic acid biosynthesis, or both. The most effective folic acid antagonists are the 4-amino derivatives of folic acid, aminopterin and α-methopterin, which prevent the conversion of folic acid to folinic acid (citrovorum factor). In this way, folic acid is prevented from acting as a formylating agent in biochemical one-carbon transfer reactions. Folic acid antagonists are especially useful in the treatment of acute lymphoblastic leukemia (*31*). The purine antagonists include 6-mercaptopurine, azaguanine, 2,6-diaminopurine, thioguanine, 6-chloropurine, and the pyrazolo pyrimidines.

A more specific attack on blocking purine metabolism has been made possible by the work of Buchanan and associates (*32*), who have characterized the enzymes and elucidated each step in the biosynthesis of purines. The antibiotics, azaserine and 6-diazo-5-oxy-L-norlucine (DON) are structural analogs of glutamine and block the enzyme that utilizes it in purine synthesis. The ability of these antibiotics to block the synthesis of nucleotides is quantitatively related to their blockading of the growth of certain types of neoplasms (*33*). Many other types of enzyme inhibitors are being investigated for possible use in cancer chemotherapy.


## Summary

The use of chemical reagents as enzyme inhibitors has yielded information concerning the mechanism of inhibition and the role in catalysis played by active groups on the protein apoenzyme, the coenzyme, or the metal component. Inhibition analysis has also furnished valuable clues concerning the architecture, chemical properties, and catalytic mechanism of the active site of the enzyme. In many cases, *in vivo* effects of inhibitors can be closely correlated with *in vitro* inhibition of purified enzyme systems. The effects of antimicrobial and anticancer agents, insecticides, and drugs can often be explained in terms of enzyme inhibition. The design and synthesis of new inhibitors offers great promise when applied to the control of undesirable organisms and to the prevention and cure of disease in the immediate future.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# J. W. Scott, Zoologist

John W. Scott was born on a farm in Lewis County, Missouri, on 1 July 1871. From the local schools he went on to graduate from the University of Missouri and later received a master's degree in psychology at the same institution. After some experience in high-school teaching he came to a major decision: he wanted to learn all that there was to know about some field of science, and he chose zoology. In taking his Ph.D. at the University of Chicago and throughout the rest of his life he was keenly interested in all branches of animal science. He could truly be called a general zoologist.

In 1913 he was appointed chairman of the department of zoology at the University of Wyoming, and his subsequent scientific interests were centered around this state and institution. He once said that he was determined to master any subject that he undertook, and he set about learning all that there was to know about Wyoming. For 40 years he walked, rode, and drove over every corner of the state, collecting, photographing and observing. With this knowledge, he helped lay the foundation for the study of animal ecology in the area, and he summarized it in a chapter in Shelford's *Naturalist's Guide to the Americas.*

To master meant to understand, and his understanding of nature included affection. In his spare time he was an ardent fisherman, and he soon became an ardent conservationist. As president of the state Izaak Walton League, he worked for the adoption of a model fish and game law and was later asked to help put it into effect as executive secretary of the State Fish and Game Commission. He was made an honorary national president of the Izaak Walton League and served as chairman for its Conservation Education Committee, which became the present Conservation Education Association, of which he was also honorary president.

His early scientific work was concerned with the embryology of the marine annelids which he studied at the Woods Hole Marine Biological Laboratory, where he was an instructor in the