BIOLOGICAL ANTAGONISM

THE THEORY OF BIOLOGICAL RELATIVITY

By GUSTAV J. MARTIN Sc.D.

Research Director

The National Drug Company

Philadelphia

PREFACE

At rare intervals into the systems of biological research come concepts which are intriguing to the scientific mind. These concepts are frequently of such simplicity as to cause wonder at their delayed arrival. Then, on closer examination, it is seen that a period of growth extending over many years led to the ultimate fully formed structure. Such a concept is the one of structural displacement, which rightly has been called "The Rational Approach to Chemotherapy."

To the immunologists goes credit for the first recognition of the value of the theory. Ehrlich applied it in his famed "Lock and Key" analogy. A more rewarding and stimulating approach to immunology had not been suggested before his time nor has one been recorded since. An entire world —the world of the antigen and antibody—grew up around this "Lock and Key" approach.

Enzyme chemists soon took up the new concept and developed it into a fundamental structure. To the embarrassment of the biochemist and pharmacologist alike, it must be admitted that research in these fields failed to recognize the merits of the metabolite analogue approach until much later.

The renaissance of the concept came with the work of Woods in England in 1940. Its popularity grew until 1947 when many early enthusiasts began to abandon the cause. The concept remains a storehouse of potential chemotherapeutic and pharmacological agents.

The purpose motivating the preparation of this summary of knowledge in the field of displacement is a belief that in no single instance of specific displacement has a thorough job been done, and that such work, properly undertaken, will lead to discoveries of chemotherapeutic agents of great value in medical science. The author feels that a reference work on the subject will facilitate the entry of others into the field and thus aid in causing the concept to be formed into a scientific structure of great practicality.

It is to be assumed that the first attempt at an outline of a concept of such great scope may result in a failure to cover adequately all works of merit. Future revisions of this volume are contemplated and all criticisms and suggestions will be gratefully received.

The author wishes to express his sincere thanks to those individuals whose cooperative spirit lightened the burdens associated with the prepa-

ration of this manuscript. He is grateful to Drs. Robert Raffauf, Werner Boehme, and J. Morton Beiler for the time they spent checking technical accuracy. Further, acknowledgment is made of the fine work done by Miss Doris Testardi and Mrs. Katherine Delsordo in the typing and proofreading of the book. He wishes to thank Mrs. Patricia Martin for her constant help and encouragement. Finally, he is deeply indebted to Mr. A. B. Collins for his sympathetic understanding and support of this work.

GUSTAV J. MARTIN

Philadelphia
August 1951

CONTENTS

Chapter 1

ENZYMATIC INHIBITION BY CHEMICALS STRUCTURALLY RELATED TO THE NATURAL SUBSTRATE

1. Specific Action of Enzymes.
2. Kinetics of Enzyme Action.
3. Kinetics of Enzyme Inhibition.
4. Succinic Dehydrogenase, Lactic Acid Dehydrogenase.
5. Acetate Metabolism; Fluoroacetate and Related Molecules.
6. Lipases, Liver Esterase, and Pancreatic Lipase.
7. Urease.
8. Certain Enzymes Involved in Carbohydrate Metabolism.
9. Miscellaneous.
10. Recapitulation.

In opening a review of metabolite analogues with a discussion of enzymatic inhibition by chemicals structurally related to the natural substrate, the purpose is to cover briefly specificity and kinetics. A consideration of the kinetics of enzymatic inhibition will serve to focus attention on the fact that the subject covers competitive and noncompetitive inhibitors, as well as those not yet classified.

Many of the displacing agents to be considered later in this book function by virtue of similarity in structure to substrate; their exclusion from this chapter is in the interests of organization and is not based upon any distinction in basic mechanism.

Specific Action of Enzymes

Enzymes possess absolute, stereochemical, and relative specificity. As an example, carboxypeptidase has absolute specificity in that it will not attack carbohydrates or fats; stereochemical specificity in that it will not hydrolyze synthetic peptides whose terminal amino acids are of the D-configuration, although the corresponding L-compounds are sensitive; and relative specificity in that it hydrolyzes carbobenzoxyglycyl-β-2-thienylalanine at only one-half the rate for the benzene relative. The study of metabolite analogues is based upon the existence of a high degree of selectivity. In fact, such study is also an approach to the determination of the degree of enzymatic

specificity. Examples of enzymatic selectivity are presented in the textbook of enzymology by Sumner and Somers (1947).

Kinetics of Enzyme Action

The inhibitory power of any given antimetabolite will be a direct function of its effect on the rate of an enzymatic reaction. Specifically, potency will be a function of the capacity of a chemical to reduce the available products of an enzyme mechanism. It must be kept in mind that no enzyme is "an island unto itself" and therefore the in vivo activity will be a function of the inhibitory action and, in some cases, stimulatory effect of a compound on a series of enzymes.

The law of mass action states that the rate of a chemical reaction is proportional to the active masses of the reacting substances. This holds for catalyzed reactions. The rate of a reaction is the speed at which the concentration of any given reacting molecule is changing at a given instant. With enzymatic reactions, there are optimal pH and temperature values which, unless specified, are assumed to be controlled and constant.

The speed or velocity of an enzymatic reaction is initially constant, then declines progressively with time due to the exhaustion of substrate, accumulation of end-products, etc. In general, in all considerations of antimetabolites, we are concerned with this initial and constant reaction rate.

For purposes of clarification, it seems wise to review the mathematical formulations for the kinetics of ordinary reactions.

First order reactions are those in which the rate of decomposition is directly proportional to concentration. The mathematical expression is:

$$-\frac{dc}{dt} = kc$$

where c is the concentration of the material, k is a proportionality factor, and $-\ dc/dt$ is the rate of change. This equation may be modified as follows:

$$k = \frac{2.303}{t} \log \frac{a}{a - x}$$

where a is the initial concentration, x is the amount reacting in time t, and $a - x$ is the concentration remaining after time t. k is the velocity constant.

Second order reactions involve two molecules and depend upon collision frequency. The mathematical expression is:

$$\frac{dx}{dt} = k(a - x)\ (b - x)$$

where a and b represent initial molar concentrations, x denotes amounts changed in time t. On integration of the equation, taking into consideration that $x = o$ when $t = o$, and that $x = x$ when $t = t$, it is seen that:

$$k = \frac{2.303}{t(a-b)} \log \frac{b(a-x)}{a(b-x)}$$

where k is the reaction constant.

The mathematical expression of a zero order reaction is:

$$-\frac{dc}{dt} = k = \text{rate of reaction}$$

Here the rate of reaction is constant over a period of time.

In enzymatic reactions, another factor—concentration of enzyme—is added. Such reactions are generally of zero or first order; however, it should be kept in mind that the order of an enzymatic reaction may change as it proceeds. Enzymatic reactions of zero order are expressed as:

$$-\frac{dC_a}{dt} = kC_e$$

where C_a is the concentration of the reactant; C_e is the concentration of the enzyme.

The first order enzymatic reaction is expressed as:

$$-\frac{dC_a}{dt} = kC_aC_e = \text{reaction rate}$$

and if C_e is constant over a short period of time, the reaction has the characteristics of an ordinary first order reaction and can be written:

$$kC_e = K' = \frac{2.303}{t} \log \frac{a}{(a-x)}$$

a and x have same significance as is given above.

Another form of this equation which is the most practical for purposes of calculation is:

$$K' = \frac{2.303}{t_2 - t_1} \log \frac{C_1}{C_2}$$

where C_1 and C_2 are substrate concentrations at times t_1 and t_2.

Second order enzymatic reactions are expressed as:

$$-\frac{dC_a}{dt} = -\frac{dC_b}{dt} = kC_eC_aC_b = \text{rate of reaction}$$

C_e, C_a, and C_b respectively are the concentrations at time t of enzyme and the reactants a and b of the second order reaction.

Zero order enzymatic reactions are those in which the concentration of the substrate is high compared to the amount of enzyme employed. This assures a constant concentration of the enzyme-substrate complex which is the reactant in this type or order of reaction. The enzyme-substrate complex is an assumption but for all practical purposes seems in order.

First order reactions vary in rate as the substrate concentration decreases and the product formed increases in amount. The rate will slow as time progresses. The action of hydrolases in dilute solution is a good example.

Whether or not second order enzymatic reactions occur is open to question. If they do, the speed varies with the product of the concentrations of the reactants.

The order of a reaction is determined by plotting graphically different functions of concentration of reactant against time and determining which one gives a straight line. If log c against time gives a straight line the reaction is of the first order, which means that the time taken for a given fraction to react is independent of the initial concentration. A second order reaction would give a straight line by plotting log $(a - x)/(b - x)$ against time or when the initial concentrations of the reactants are equal, by plotting $1/c$ against time. In a zero order reaction, plotting c directly against time would give a straight line, indicating a constant rate of reaction over at least a short interval of time.

As the order of enzymatic reactions may shift, it is advisable from a practical standpoint to consider only the initial rate. At this stage, concentrations of reactants and enzyme are more stable and the reverse phase which is a characteristic of most enzymatic reactions has not yet come to play a significant part. Enzyme concentrations in these mathematical formulations are expressed in terms of their activity.

Neurath and Schwert (1950) have presented an analysis based on kinetics of inhibitor action which considers the concentration of the enzyme-substrate complex and therefore differs slightly from the equations presented above. The formulation for enzymatic action represents an extension of the concepts of Michaelis and Menten (1913) and is expressed as follows:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} (ES) \xrightarrow{k_3} E + P$$

E = free enzyme. S = free substrate. ES = enzyme-substrate complex. P = reaction products. k_1, k_2, and k_3 = rate constants.

The rate of the overall reaction is represented as:

$$d(p + a)/dt = -k_3 p$$

where p and a represent concentrations of enzyme-substrate complex and free substrate. For a zero order reaction this reduces to a modified form the equation for the zero order enzymatic reaction given above, namely:

$$-da/dt = k_3 p$$

In other words, the rate is proportional to the concentration of the enzyme-substrate complex. In the system under consideration, the concentration of

the enzyme-substrate is constant which leads to the mathematical formulation:

$$\frac{k_2 + k_3}{k_1} = \frac{(e - p)a}{p} = \frac{(E)\ (S)}{(ES)} = K_m$$

From this equation it is possible to derive an expression for reaction velocity in terms of enzyme and substrate concentrations:

$$v = -\,da/dt = \frac{k_3 ae}{K_m + a}$$

Still another mode of presentation of velocity rate is that in which all of the enzyme is bound in an enzyme-substrate complex. This then represents a maximum velocity, V_{max}, and the equation becomes:

$$v = \frac{V_{max}\, a}{K_m + a}$$

A linear relation between v and a is obtained from the equation:

$$\frac{1}{v} = \frac{K_m}{V_{max}} \cdot \left(\frac{1}{a}\right) + \frac{1}{V_{max}}$$

Kinetics of Enzyme Inhibition

For presentation purposes, Neurath and Schwert (1950) presented a mathematical kinetic representation of the inhibition of proteolytic reactions. For the competitive type of inhibition, it is assumed that both inhibitor and nutrilite compete for the same reaction site on an enzyme surface. This can be represented as follows:

$$E + S \rightleftarrows ES \rightarrow E + P$$
$$E + I \rightleftarrows EI$$

I and EI indicate inhibitor and enzyme-inhibitor complex concentration. The degree of inhibition depends on the concentrations of ES and I and can be expressed as:

$$\frac{1}{v_i} = \frac{1}{V_{max}} + \left[1 + \frac{(I)}{K_i}\right] \frac{K_m}{V_{max}} \frac{1}{a}$$

V_i = initial velocity in the presence of the inhibitor.
K_i = dissociation constant of the enzyme-inhibitor complex.
I = concentration of inhibitor.

The noncompetitive type of inhibition assumes reaction of inhibitor with catalytically inactive sites on the enzyme surface and is expressed as follows:

$$ES + I \rightleftarrows (ESI)\ \text{inactive}$$

and mathematically as:

$$\frac{1}{v_i} = \left[1 + \frac{(I)}{K_I}\right]\left[\frac{1}{V^i_{max}} + \frac{K_m}{V^i_{max}}\frac{1}{a}\right]$$

V^i_{max} = maximum velocity in the presence of an inhibitor.

There are instances in which the action of an inhibitor may be noncompetitive at one concentration and become competitive as the concentration increases and the inhibitor begins to be bound at the catalytically active enzyme surface site.

Hunter and Downs (1945) presented a mathematical expression of the kinetics of inhibitors which assumed a negligible fraction of total inhibitor present combined with enzyme. For a noncompetitive system, the formulation was:

$$I\frac{a}{1-a} = K_I$$

K_I = dissociation constant of enzyme inhibitor complex.
I = concentration of inhibitor.
a = fractional activity of enzyme.

This equation shows that with given enzyme and inhibitor concentrations, the fractional activity is constant and independent of substrate concentration. The controlling factor is the degree of dissociation of the enzyme-inhibitor complex.

For competitive inhibition, the Hunter and Downs equation is:

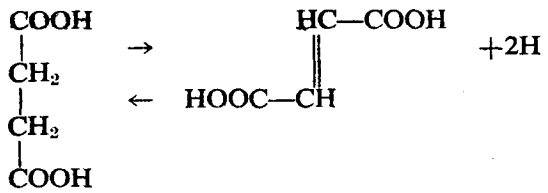
$$I \cdot \frac{a}{1-a} = K_I + \frac{K_I}{K_s} \cdot S$$

S = substrate concentration.
K_s = dissociation constant of enzyme-substrate complex.

The equation shows that the extent of inhibition is proportional to the relative values of the dissociation constants of the enzyme-inhibitor and enzyme-substrate complexes and to inhibitor and substrate concentrations. The effectiveness of a given competitive inhibitor depends upon its concentration and relative affinity for the enzyme as compared to the substrate.

Succinic Dehydrogenase, Lactic Acid Dehydrogenase

Succinic dehydrogenase is one of a group of enzymes possessing the ability to reduce cytochrome C. Recent evidence indicates that this might not be a direct mechanism. The enzyme converts succinic acid to fumaric acid provided a hydrogen acceptor is present. Under aerobic conditions the hydrogen goes to oxygen by the action of cytochrome-cytochrome-oxidase.



Succinic acid **Fumaric acid**

This transformation is part of the tricarboxylic acid (Krebs) cycle in which oxalacetic acid condenses with acetic acid in a chain of reactions which eventually give rise to α-ketoglutaric acid and carbon dioxide. α-Ketoglutaric acid is then decarboxylated to succinic acid and it is at this point that succinic dehydrogenase functions in bringing about the next step to fumaric acid. The final phase is regeneration of oxalacetic acid which partakes in another cycle.

Quastel and Wooldridge (1928) first studied the inhibition of succinic dehydrogenase by substrates structurally related to succinic acid. Malonic acid, which is the lower homologue of succinic acid, blocked the action of the enzyme by preventing access of succinic acid to the reaction site. The mechanism was that of purely competitive inhibition as demonstrated by Hopkins et al. (1938) who found that adequate succinic acid completely overcame the antimetabolite action. In addition to malonic acid, Quastel and Wooldridge found an entire series of carboxylic acids to be effective inhibitors (Fig. 1).

In the inhibition of succinic dehydrogenase by malonic acid, the affinity of antimetabolite and enzyme is so great that the inhibitor/substrate ratio for 50 per cent inhibition is 1/50 (Potter and DuBois, 1943). This is an exceptional case and represents one of the few instances in which K_I is lower than K_S.

Krebs and his coworkers (1937, 1940, 1940a, 1940b) used malonic acid as an important tool in establishing the citric acid cycle. They found that fumaric acid removes the malonate inhibition of pyruvate oxidation; succinic acid accumulated under the conditions of the experiment. Again, in the presence of malonic acid, they found that fumaric disappears and succinic accumulates even if no pyruvate is added.

Other compounds known to inhibit succinic acid dehydrogenase are: oxalic, adipic, aspartic, malic, fumaric, and oxalacetic acids (Bandhu, 1937; Potter and Elvehjem, 1937).

Of a series of alkylated malic, succinic, and malonic acid derivatives, Franke (1944) found that the alkylmalonic acids did not inhibit succinic dehydrogenase. The alkyl succinic acids from the octyl to the dodecyl compounds were inhibitors; the alkyl malic acids were not. In general, it would seem that the carbon alkyl acids of this type are either inactive or weak

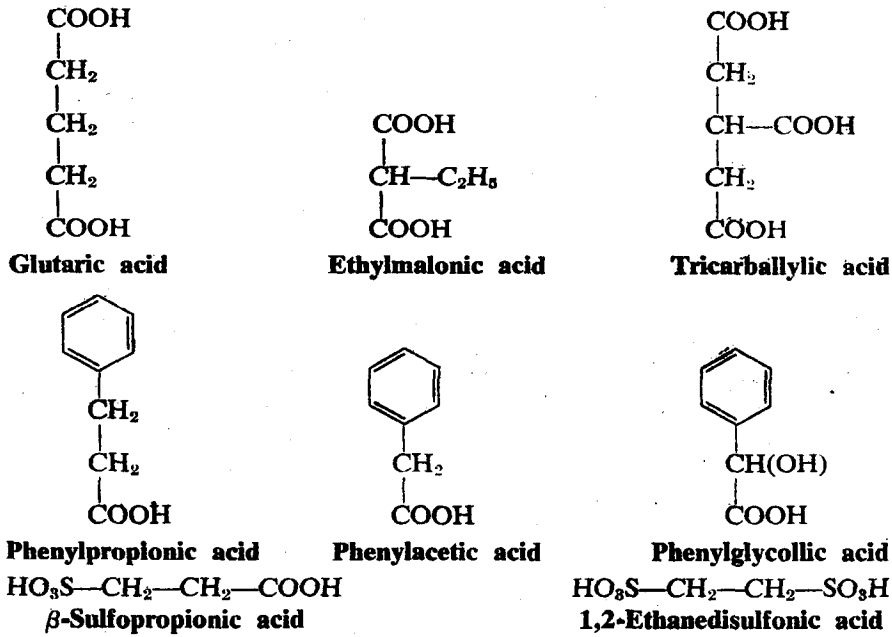


FIG. 1. Metabolite antagonists of succinic acid.

inhibitors. The sulfonic acid analogues of succinic acid—β-sulfopropionic acid and 1,2-ethanedisulfonic acid—are both effective antimetabolites with a potency roughly equivalent to that of malonic acid (Klotz and Tietze, 1947). The competitive or noncompetitive nature of the action was not determined but it seems probable that as with so many other sulfonic acid analogues the activity would be nonspecific.

Recently, Pardee and Potter (1949) discovered the blockage of oxalacetate oxidation by malonate, a phenomenon partially dependent upon the concentration of magnesium ions. The formation of a complex of malonate with free and bound magnesium was offered in explanation of the findings. This would probably be a chelation mechanism, which seems to assume more and more importance in biological relativity. They do not imply that magnesium chelation (complex formation) underlies the entire blockage process but they do regard it as an important factor.

Again, the succinoxidase system has been employed by Ackermann and Potter (1949) to extend the differential metabolite requirement theory (Martin, 1944) to the equivalent of a differential enzyme concentration-inhibitor concept. This basic idea, a manifestation of the biological relativity theory, proposes that the effect of a given inhibitor in vivo will be greatest for the tissue containing the inhibited enzyme in the lowest concentration. Ackermann and Potter use as an example of the application of

this concept the production of alloxan diabetes (see purine and pyrimidine section, p. 382). They point out that alloxan is a general sulfhydryl inhibitor and that selective action against the insulin-producing cells is therefore unlikely. The only logical alternative would be that these cells are more vulnerable because quantitatively they lack a vital enzyme, plentiful in other tissues.

The main point made in their presentation is that the so-called irreversible enzyme inhibitors, such as copper in the case of succinic dehydrogenase, exert their action in proportion to the concentration of the enzyme. In a complex in vivo system, the effect of the irreversible inhibitors will be a function not only of the enzyme concentration but also of the concentration of other substances which will combine with the inhibitor. This is the basis of the observed reversal of the action of copper on succinoxidase by glutathione.

There have been many other studies of succinic acid analogues and comparatively few of them can be discussed in the interests of brevity, but there is one—namely, trans-1,2-cyclopentanedicarboxylic acid—which brings into consideration another factor in the study of displacement. Seaman and Houlihan (1950) have reported that this compound increases the permeability of the membrane of *Tetrahymena geleii* to succinate. Normally, the membrane is impermeable to succinic acid. The in vivo action of this molecule, in itself an inhibitor of the oxidation of succinate, should differ materially from that of a compound capable of inhibiting in vitro but without a similar action on permeability. The alteration of membrane permeability by metabolite analogues may well be a function of their inhibitory capacity or it might be dissociated therefrom. Research along these lines offers hope for discoveries in the field of membrane permeability.

Another study of general importance bearing on this subject is that of Schulman and Armstrong (1949, 1949a). They tested a series of compounds of the type $X(CH_2)_nY$ for activating or inhibiting power in the decolorization of methylene blue by yeast cells or by succinic dehydrogenase. With this enzyme, the order of decreasing inhibitory power is as follows:

$$X,Y;\ -CH(NH_2),-NH^+;\ H,\ CH(NH_2)NH^+;\ H,\ NH_3^+;\ H,\ SO_4^=;\ H,\ CO_3^=;\ H,\ NMe_3^+;\ H,\ SO_3^=;\ H,\ \text{bile salt.}$$

The same compounds activated the yeast cell systems and the activation probably is due to the increased permeability of the cell membrane for the methylene blue. Here, again, the factor of membrane permeability plays a vital role.

These investigators (Schulman and Armstrong, 1949a) extended their consideration of the permeability factor and report that only compounds with one long substituted chain in a series of alkyl substituted succinic acids

and half esters activate the decolorization of methylene blue by yeast cells.

Finally, the noncompetitive inhibition of succinic dehydrogenase by mononucleotides (Zittle, 1946) appears to confuse the picture until it is viewed in the light of the energy transfer of the dehydrogenase mechanism to high potential phosphate bonds such as those of adenosine triphosphate, phosphocreatine, and enol phosphate (Colowick et al., 1941). This cross link on an enzymatic system may represent another type of inhibition, in that the mononucleotide competitively inhibits a system which receives energy from the dehydrogenase; blocking this energy transfer retards the dehydrogenase.

Coincident with their work on succinic dehydrogenase, Quastel and Wooldridge (1928) studied the inhibition of the dehydrogenation of lactate to pyruvate (lactic acid dehydrogenase) and found that the reaction was blocked by an entire series of molecules, including α-hydroxybutyric, glyceric, mandelic, glyoxylic, and oxalic acids. Malonic acid was subsequently found to exert a similar action (Das, 1937).

Acetate Metabolism; Fluoroacetate and Related Molecules

One of the first things that comes to mind in a consideration of fluoroacetate is the biological activity of its relative, iodoacetate. Iodoacetate is known to inactivate or inhibit choline acetylase (Nachmansohn and Machado, 1943), papain (Fruton and Bergmann, 1940), yeast alcohol dehydrogenase (Dixon, 1937), isocitric dehydrogenase, enolase (Meyerhof and Kiessling, 1935), 1,3-diphosphoglyceric aldehyde dehydrogenase (Adler et al., 1938), and aldehyde mutase (Dixon, 1938–39). Its function seems to be that of reacting with sulfhydryl groups of the enzyme protein, causing inactivation. Its effect is therefore nonspecific and it will block any enzyme requiring intact —SH for activity. This is mentioned to emphasize the fact that there is no biochemical, physiological, or pharmacological similarity between iodoacetate and fluoroacetate action.

Attention should be directed to the basic mechanism of action of fluoroacetate, which is that of interference with acetate metabolism by prevention of the formation of an "active" acetate (Bartlett and Barron, 1947). Furthermore, fluoroacetate has been isolated from the South African plant, *Dichapetalum cymosum,* by Marias (1944). The plant, which was known locally as "Gifblaar," had long been recognized as toxic to livestock. This is another example of a "synthetic" antimetabolite which is also found in nature.

The studies that have established the displacer activity of monofluoroacetate are too numerous to be reviewed here. An excellent and detailed summary of this information is contained in an article by Chenoweth (1949). It is indicated, however, that some of the background work be considered.

Many test systems have been used in studies of fluoroacetate. One of these, which is pharmacologically significant in addition to being a reflection of basic mechanism, involves the use of intestinal smooth muscle. Weeks et al. (1950) found that both sodium dehydroacetate and fluoroacetate interfere with the maintenance of spontaneous contractility in intestinal strips. The toxic effect of both displacers was counteracted by glucose and sodium acetate. Similar findings were simultaneously reported by Farah et al. (1950). Pyruvate and some of the even numbered carbon fatty acids, as well as acetate, were able to sustain contractions in the normal muscle but failed when sodium fluoroacetate was added. There was a quantitative relationship between the concentrations of sodium acetate and fluoroacetate which would produce a certain degree of inhibition, a fact which was interpreted to indicate competitive mutual antagonism. Another facet of their observations concerned the fluoroacetate sensitive and resistant contractions. The proposed explanation was that two pathways of energy supply for contraction exist; the sensitive reaction via pyruvate and acetate, the other via a pathway involving neither the Krebs cycle nor the cytochrome system. It seems probable that maintenance of contractility might be effected through the addition to the system of phosphocreatine or adenosine triphosphate as Colowick et al. (1949) have demonstrated a failure of oxidative resynthesis of phosphocreatine by frog muscle poisoned by methyl fluoroacetate.

In the isolated heart, similar effects of fluoroacetate are noted. Either acetate or glucose in the perfusate will maintain contraction and protect the rabbit and monkey heart against fluoroacetate (Chenoweth, 1949). The molar ratio for protection was approximately unity. Pyruvate is less effective than acetate as a biochemical antidote for fluoroacetate (Braun-Menendez et al., 1939).

The resting potential of frog nerve is preserved by the formation of pyruvate and its subsequent aerobic metabolism. Methylfluoroacetate blocks the recovery of resting potential of nerve in oxygen following anoxia (Shanes, 1949), an action prevented by sodium pyruvate. Sodium fluoroacetate is not active in isolated frog brain preparations (Brooks et al., 1949).

Mechanism studies of the action of monofluoroacetate in intact animals have revealed that sodium acetate is an effective antidote (Tourtellotte and Coon, 1949). Ethanol was also active, and a combination with acetic acid suggested synergism. Confirmation of this work came from Hutchens et al. (1949) and its subsequent extension revealed the effectiveness of other two carbon moieties, particularly glycerol monoacetate (Chenoweth et al., 1949a).

Studies of tissue slices, homogenates, and microörganisms have revealed that the basic action of fluoroacetate is essentially similar throughout. The

first such study was that of Bartlett and Barron (1947) who found that fluoroacetate had no effect on sulfhydryl enzymes. In the presence of the compound in an isolated tissue, acetate accumulated in the presence of pyruvic acid, the oxidation of fatty acids and the formation of acetoacetic acid was blocked, and carbohydrate synthesis from acetate or pyruvate was stopped. Acetylation reactions were not prevented when sulfanilamide, p-aminobenzoic acid, and choline were substrates. This is an important example of the selectivity of antimetabolites in the blockage of one channel of substrate metabolism with no effect on another. In 1948, Kalnitsky and Barron published the results of their extended studies. Using fresh rabbit kidney homogenates, they observed the inhibition by fluoroacetate and fluorobutyrate of the oxidation of caproic, acetic, butyric, pyruvic α-ketovaleric and α-ketocaproic acids, and glucose. Ethyl alcohol released the fluoroacetate inhibition of acetic acid oxidation.

Again, with microörganisms, fluoroacetate completely inhibits the oxidation of yeast (Kalnitsky and Barron, 1947). If the acetate is added before the inhibitor, there is no blockage. This is clear evidence supporting the competitive nature of the phenomenon. Neither the other halogen acetates nor trifluoroacetate had this activity. The inhibition occurs in the first step of acetate metabolism, as shown by inhibition of citric acid synthesis from acetate and the accumulation of acetate during the oxidation of glucose or ethanol. Black and Hutchens (1948) have studied the time element in the fluoroacetate-acetate system. If the inhibitor was added to yeast 25–30 minutes before the acetate, no significant oxygen uptake occurred until an extended induction period (approx. 3 hrs.) had elapsed. During this period, the addition to the system of ethanol, acetaldehyde, succinate, and succinic semialdehyde catalyzed the oxidation.

An observation such as that of Fitzgerald et al. (1949) on the inhibition by iodoacetate and fluoroacetate of the adaptive enzyme formation in *Mycobacterium lacticola* probably represents a nonspecific phenomenon, not associated with direct competitive displacement of acetate.

In addition to the nonspecific physiological activity, it is logical to expect certain related molecules to act by conversion to fluoroacetate. This is the case with fluoroethanol (Bartlett, 1949), which functions entirely by virtue of oxidation to the corresponding acid and in itself possesses no activity. In fact, Saunders (1947) concluded from the study of the toxicity of a series of homologous compounds of the type $F(CH_2)nCOOR$ that only those members were toxic which could give rise to fluoroacetate by β-oxidation.

The pharmacological and toxicological characteristics of monofluoroacetate have been reviewed in detail by Chenoweth (1949) and it is clear that certain information presented in his review correlates with the hypothesis of Ackermann and Potter (1949) that any given antimetabolite will

function as a toxic agent in a specified system in accordance with the relative importance of the affected enzyme to that system. The toxicity of fluoroacetate varies with species from an LD_{50} of 0.06 mg./kg. in the dog to over 500 mg./kg. in a species of toad. Here, the experimental findings would indicate that the enzymatic mechanisms blocked by fluoroacetate were roughly 10,000 times more important to the dog than to the toad, a good working example of the theory of biological relativity. Again, the major point of attack on specific tissues varies; it may be the central nervous system, the heart, or both. The organ or tissue affected is that one to which the blocked enzymatic reactions are the most vital—the tissue containing the lowest concentration of the inhibited enzyme. It is interesting to speculate on the reason for cardiac manifestations in herbivorous animals, and central nervous system effects in carnivores.

Lipases, Liver Esterase, and Pancreatic Lipase

The lipases are a subgroup in the general classification, esterases. All esterases catalyze the reversible reaction:

$$\underset{\text{Ester}}{R'\text{—O—OC—R}} + H_2O \rightleftarrows \underset{\text{Alcohol}}{R'OH} + \underset{\text{Acid}}{R\text{—COOH}}$$

The specificity of lipases has been the subject of study by Weinstein and Wynne (1935–36) who found that the initial rates of hydrolysis of some triglycerides have the following order: tripropionin > tributyrin > tricaproin > triacetin > trivalerin. While the pancreatic lipases will hydrolyze simple esters, liver esterase is relatively specific for these molecules, especially ethyl butyrate. Liver esterase will act on fats, but very slowly.

Murray and King (1930) first investigated the relative affinities of pairs of optically active secondary alcohols for liver esterase by observing the extent to which their presence inhibits the hydrolysis of ethyl butyrate or ethyl propionate. The L-forms of methyl-n-hexylcarbinol, methylphenylcarbinol, and methyl-β-phenylethylcarbinol inhibit sheep liver esterase 4–5 times more powerfully than the D-forms. With rabbit liver esterase, the inhibition by both D- and L-forms was about the same. The optical antipode specificity of the sheep liver esterase is understandable but the lack of similar selectivity by rabbit liver esterase is not.

The study of the hydrolysis of asymmetric esters by enzymes led Bamann and Laeverenz (1931) to the observation that liver esterase hydrolyzes more rapidly the D-component in a DL-mandelic acid ester composition. When the optically active forms were used separately as substrates, the L-component was more susceptible to the action of the enzyme. The implication of this work is that the D-component in the mixture inhibited the esterase action on the L-isomer and itself underwent hydrolysis.

The relationship between structure of aliphatic alcohols and their inhibi-