Enzyme Mechanism

The manipulation of biological systems is discussed at an enzymatic level.

Steven Benner

In 1813 Berzelius wrote, "The cause of the most phenomena within the animal body lies so deeply hidden from our view that it certainly will never be found. We call this hidden cause, *vital force*." This statement was written well before the birth of modern biological science. Berzelius was one of the leading scientists of the time, and the mysterious "vital force," something presumed to be unrelated to physical or chemical laws, was considered the most fundamental principle underlying living systems. According to "vitalists," a living system could only be treated as a "black box" which took in food and oxygen and produced from these substances the characteristics of life. Exactly how the food and oxygen were converted to life was regarded as unknowable.

The ultimate repudiation of vitalism occurred gradually as researchers discovered in physical and chemical terms more about how life operated. The concept of mechanism, the focus of this article, was crucial in this repudiation. By mechanism we mean the set of discrete steps taken in a system (e.g., a man or an automobile) to convert observable input (food or gasoline) into observable output (life and .motion). Understanding mechanism is important when someone (e.g., a physician or a mechanic) wishes to ' manipulate a system, to make it run

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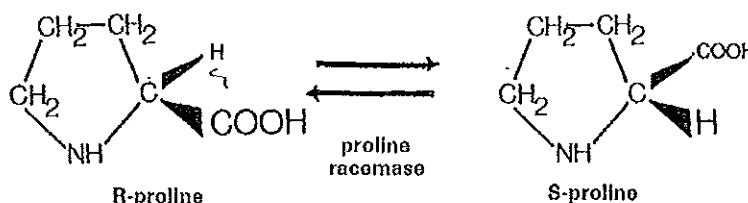

Fig. 1. Interconversion of S- and R-proline by proline racemase.

more efficiently, or to repair it when it breaks down. What would happen, for example, if a mechanic tried to repair a car without understanding how it works? He might add more gasoline or try different types of motor oil. He might open the engine and arbitrarily adjust parts. But no matter what he does, his actions will be essentially random. The car may indeed be repaired, but this repair will be the result of good luck and not of rational analysis. Furthermore, the car will probably be repaired only after considerable time is expended in the process of trial and error.

Similar problems are encountered when a physician attempts to treat a patient. In certain instances, such as with broken bones, the problem is well understood and therefore easily treated. However, in more serious diseases (e.g., cancer or mental illness), where the malfunction is a fundamental alteration of biochemical pathways and control systems, very little information is available concerning the mechanism by which these diseases affect an organism. In these cases a physician, in his attempt to develop therapeutic drugs, can scarcely be more rational than our non-mechan-

istically oriented mechanic. Most of the drugs that are available to treat such diseases have been discovered accidentally by screening large numbers of chemicals chosen virtually at random.

Not surprisingly, much of current biomedical research is directed specifically towards discovering the mechanistic information needed for a more rational treatment of disease. There are many levels of complexity at which one can investigate biochemical mechanisms. However, because all observable outputs of a living system are based ultimately on the actions of enzymes, the versatile catalysts that mediate virtually every biochemical process, the mechanism of living systems must ultimately be understood in terms of the mechanism by which the enzymes themselves act.

An enzyme takes a starting material, or substrate (S), and produces a product (P). An example is the reaction catalyzed by the enzyme proline racemase, an enzyme that interconverts S proline, an amino acid, and its mirror image, R proline, drawn in perspective in Fig. 1. R and S proline are a pair of optical isomers, compounds with the same structural formula but with atoms arranged in mirror image
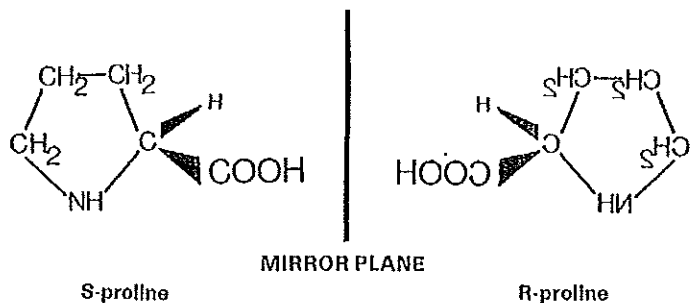
MIRROR PLANE

S-proline   R-proline

Fig. 2. S- and R-proline are optical isomers and therefore mirror images of each other.
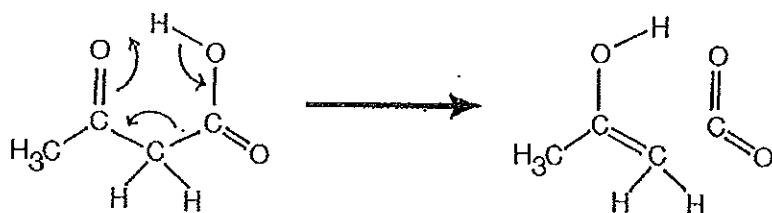

Fig. 3. Loss of carbon dioxide (CO_2) from acetoacetic acid to illustrate the movement of electrons in a chemical reaction mechanism.

configurations. That R proline is in fact the mirror image of S proline can be demonstrated by mentally rotating S proline 180 degrees (Fig. 2). Optical isomers are not identical in biological systems and enzymes can distinguish one isomer from the other. For example, all proline found in proteins is in the S isomer form.

The overall reaction catalyzed by proline racemase, like most enzyme-catalyzed reactions, relatively straight-forward. However, as with an automobile, knowlege only of inputs and outputs is not sufficient should one wish to manipulate the system; the biochemist must possess detailed knowl-edge of the mechanism of enzyme ca-talysis before he can rationally attempt to alter an enzyme's functioning.

An explanation of enzyme mecha-nisms must start with an explanation of chemical reaction mechanisms in gen-eral. A chemical reaction is the break-ing of old bonds and the formation of new bonds between atoms. Since a chemical bond is a pairing of electrons between two atoms, a chemical reac-tion may be considered as a shifting of electrons. The breaking of a bond can therefore be shown as an arrow indicating the movement of an electron pair from an old position to a different position to form a new bond. A nega-tive charge is a pair of electrons not involved in bonding. An atom originally negatively charged can become non-charged (neutral) by donating these electrons to form a new bond. Similar-ly, a non-charged atom becomes posi-tively charged if it donates a pair of its electrons to form a new bond.

A reaction that illustrates the use of arrows to describe a mechanism is the loss of carbon dioxide (CO_2) from acetoacetic acid, the compound shown in Fig. 3. Notice that the posi-tions of the atoms in the products are not distinctly different from those in the starting material. However, due to the shifting of electrons, two mole-cules finally replace the original mole-cule and one, carbon dioxide, can escape from the reaction mixture.

In studying reaction mechanism an important concept is the transition state, which is best understood in terms of the relative amounts of energy contained by the reactants, intermedi-ate states, and products of a reac-tion. These energy "levels" are shown graphically in Fig. 4. The energy at any point along the "reaction coordinate" (the degree to which the reaction has progressed) is represented by how far "uphill" that point is. The transition state is defined as the configuration of the reactants at the peak of the energy hill. The distance labeled E_a is the reaction's "activation energy,"

which signifies the amount of energy that must be added to the reactants to boost them through the transition state to become products. In the preceding example the transition state might be represented as the point at which the bonds involved in the reaction are half made or half broken (Fig. 5). The larger the activation energy (the bigger the "hill" the reactants must cross to become products) the slower the reac-tion will proceed.

A catalyst, be it an enzyme in an organism or an inorganic compound in an antipollution device, speeds up reactions. It does this by lowering the energy barrier that reactants must cross to become products. In other words, a catalyst can act by stabilizing, or lowering the energy of, the reac-tion's transition state. The stabilization of transition states and the accelera-tion of biochemical reactions in an organism are essential to life. In most organisms the body temperature is less than 30 degrees centigrade, a temper-ature so low that most reactions of bio-chemical importance would proceed very slowly or not at all in the absence of catalysis.

Several mechanisms have been sug-gested to explain how enzymes accel-erate chemical reactions. One theory, originally proposed by Linus Pauling, accounts for enzyme catalysis by pos-tulating that an enzyme stabilizes the reaction's transition state by binding more tightly to the transition state than to its substrates. The tighter binding results in the lowering of the transition state's energy by an amount repre-
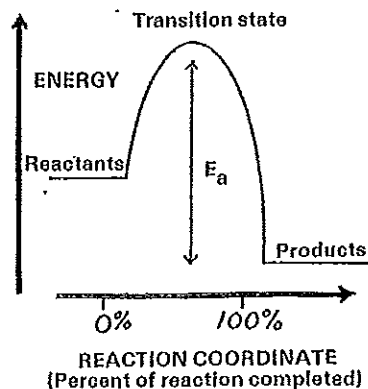

REACTION COORDINATE
(Percent of reaction completed)

Fig. 4. The relative energies of the reac-tants, products, and intermediates of a reaction are represented by how far up the energy axis each is. The transition state is the peak of the energy hill.
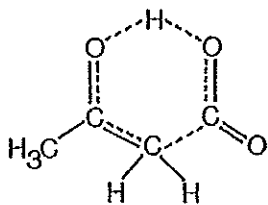
Fig. 5. Transition state for loss of carbon dioxide from acetoacetic acid. Half-formed bonds are represented by dotted lines.
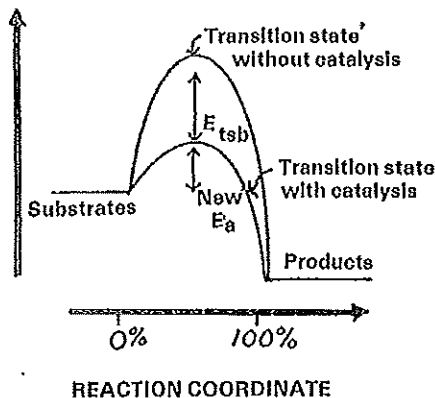

REACTION COORDINATE

Fig. 6. Tighter binding of the transition state to the enzyme lowers the energy of the transition state by E_{tsb} and therefore speeds the reaction.

sented as E_{tsb} (transition state binding energy) in Fig. 6. The degree to which the transition state is stabilized—the magnitude of E_{tsb}—is directly related to the enzyme's ability to accelerate a biochemical reaction. A general model for the mechanism of an enzymatically catalyzed reaction therefore consists of: 1) the substrate binding to the active site of the enzyme; 2) the enzyme-substrate complex moving along the reaction coordinate to become a tightly bound enzyme-transition state complex; and 3) the pro-

ducts being released from the enzyme.

With this understanding of enzyme mechanism several rational approaches could be utilized to inhibit or otherwise manipulate an enzymatically catalyzed reaction. One approach commonly used has been to design and synthesize a substrate "analog," a compound that "looks like" the enzyme's substrate but which cannot undergo the reactions of the substrate. The hope is that by imitating the substrate the analog will "fool" the enzyme and bind to the enzyme's catalytically important area (the "active site"). In this way, the active site will be blocked by the substrate and the reaction prevented. The enzyme is therefore inhibited. The effectiveness of this type of inhibitor is determined by the strength with which it binds to the active site, which in turn depends on how faithfully the inhibitor mimics certain key features of the substrate. Methotrexate, a substrate analog for dihydrofolic acid (Fig. 7) In a reaction catalyzed by the enzyme dihydrofolate reductase, binds extremely tightly to the reductase, and therefore is a very effective, inhibitor of the enzyme. As a result, the drug is used extensively in cancer therapy, since rapidly dividing cancerous cells need dihydrofolate reductase to synthesize precursors for deoxyribonucleic acid (DNA).

In many biological systems, however, an inhibitor's effectiveness is not the only criterion for its usefulness. Another important criterion is selectivity—how effectively an inhibitor specifically singles out and inhibits its target enzyme without also inhibiting other enzymes. Substrate analogs would not be expected to be very specific for their target enzyme. If a compound is used as a substrate by many different enzymes in a biological

organism, an analog of that substrate would not be expected to selectively "fool" and inhibit one of those enzymes while having no effect on the rest.

Transition State Analogs

An alternate approach to inhibitor design is predicated upon knowledge of the transition state's structure in an enzymatically catalyzed reaction. The goal of this approach is to design an inhibitor that is a transition state analog, i.e., a compound composed of atoms arranged in a configuration resembling the configuration of atoms in the tightly bound transition state. Some inhibitors of the enzyme proline racemase are good examples.

The mechanism by which R-proline is converted to S-proline can be pictured as one hydrogen coming in from the top side of the molecule and the hydrogen originally bound to the molecule leaving from the bottom side (Fig. 8). As a result the configuration of atoms around the critical carbon atom (the "alpha carbon," labeled with an asterisk) is inverted. It is important to note that in both the substrate and the product the atoms bound to the alpha carbon are arranged in three dimension in a tetrahedral configuration. In the transition state, however, the COOH and ring atoms all lie in a plane, with the leaving hydrogen below the plane and the entering hydrogen above the plane.

Substrate analogs for proline racemase might be compounds I and II (Fig. 9). In these compounds the nitrogen is replaced by a sulfur of an oxygen atom. In both bases, the alpha carbon is tetrahedral. In contrast, a transition state analog might be a compound such as III. The two double bonds in conjugation (separated by a
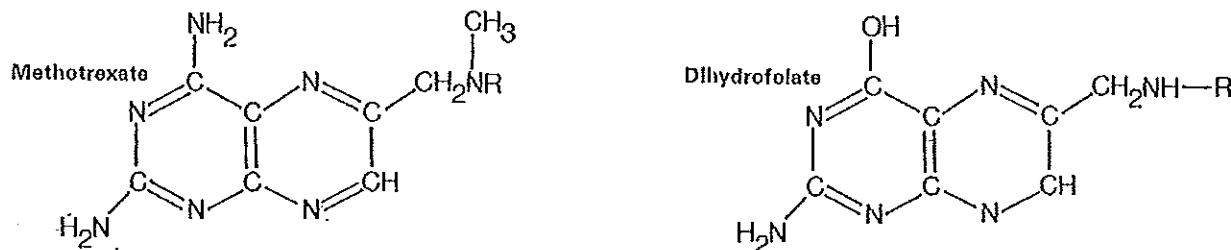

Fig. 7. Methotrexate is an effective substrate analog for dihydrofolate in the enzyme dihydrofolate reductase.
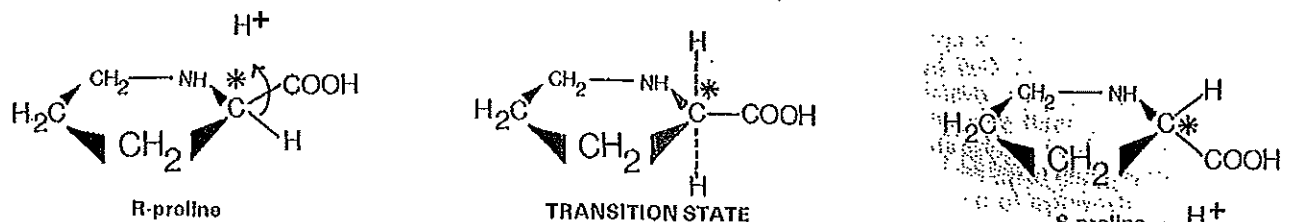
R-proline | TRANSITION STATE | S-proline

Fig. 8. In both the reactants and the products of the reaction, the asterisked carbon is tetrahedral. In the transition state, the NH, CH$_2$, and the COOH lie in a plane.
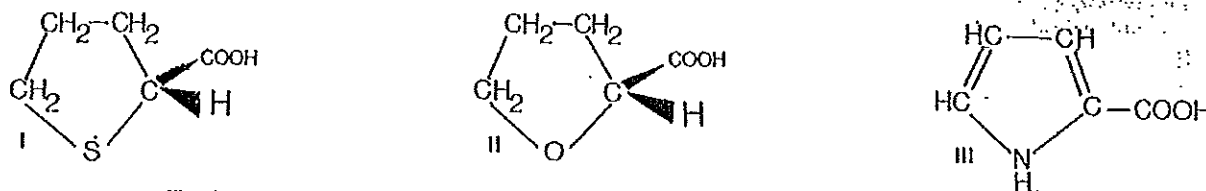

Fig. 9. Two substrate analogs and one transition state analog for proline racemase.

single bond) make the alpha carbon planar. Therefore, the configuration of the atoms of compound III resembles the configuration of atoms at the transition state of the enzymatically catalyzed reaction.

There are two advantages to using transition state analogs rather than substrate analogs in this case. First, a substrate analog for proline might be expected to mimic proline sufficiently well to inhibit enzymes other than proline racemase that use proline as a substrate. There are many such enzymes. On the other hand, by incorporating features of the transition state configuration as well as the substrate configuration into the inhibitor structure, the transition state analog would most likely affect only those enzymes that transform proline into products via a planar transition state. There is only one enzyme (the target enzyme) that does this. Therefore a transition state analog would be more specific than a substrate analog.

In addition, the transition state is more tightly bound to an enzyme than to the substrates, and thus transition state analogs would be expected to more tightly bound to the target enzyme than to substrate analogs. As a result, one would predict that transition state analogs would be much more effective as inhibitors of enzyme catalyzed reactions than substrate analogs. This is in fact the case. Transition state analogs such as III bind several orders of magnitude more tightly to the active site of proline racemase than do the substrate analogs I and II.

This example illustrates an approach using the knowledge of mechanism as the basis for rationally manipulating enzymes. This approach might be used to design drugs for therapeutic use as well, once the mechanism of disease is better understood on a macromolecular (enzymatic) level.

The transition state analog approach is currently being used in the laboratory of Dr. James Coward, Associate Professor of Pharmacology at Yale, to design potential inhibitors of several enzymes. In one case, the extra specificity available with a transition state analog is particularly important.
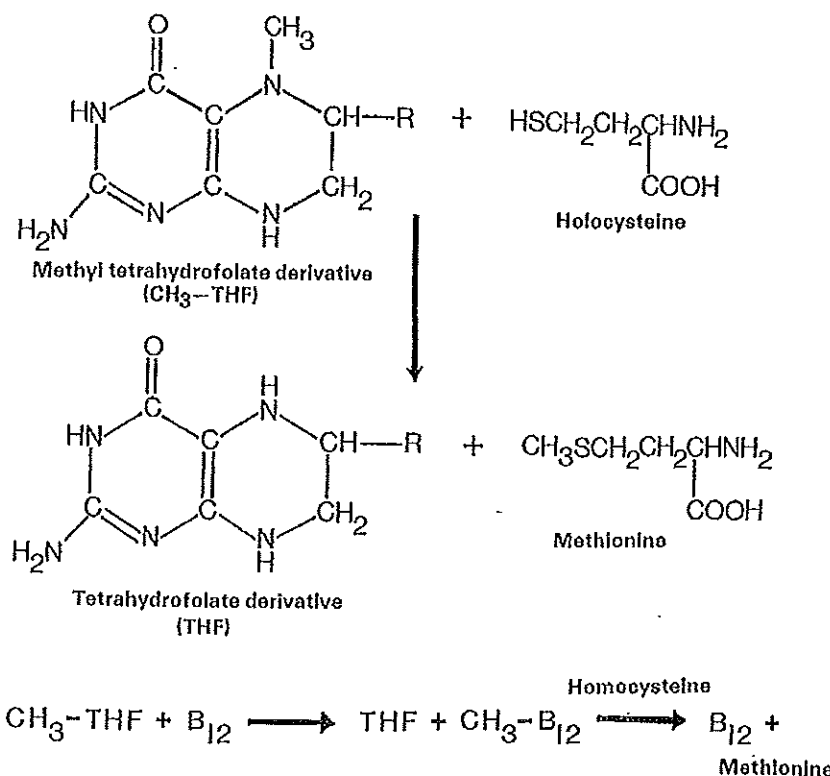

Methyl tetrahydrofolate derivative (CH$_3$—THF) + Holocysteine

Tetrahydrofolate derivative (THF) + Methionine

$$CH_3\text{-}THF + B_{12} \longrightarrow THF + CH_3\text{-}B_{12} \longrightarrow B_{12} + \text{Methionine}$$

Homocysteine

Fig. 10. The synthesis of methionine in E. Coli follows this overall reaction. In the vitamin B$_{12}$ enzyme, there is a methyl B$_{12}$ intermediate.

Methionine, an amino acid, is synthesized in the bacterium *E. Coli* by two different enzymes. The overall reactions catalyzed by both enzymes are virtually identical. They involve the transfer of a methyl group (CH_3-) from a tetrahydrofolate derivative to homocysteine (Fig. 10). One enzyme requires vitamin B_{12} to effect this transfer; the other does not. It is of biochemical interest and possibly of clinical interest as well to be able to selectively inhibit the non-B_{12} enzyme and not inhibit the B_{12}-containing enzyme. Since the substrates of the two enzymes are identical, a substrate analog could not achieve such selectivity. The mechanisms of the two enzymes are clearly different, however, because one requires B_{12} for catalysis and the other does not. Thus, selective inhibition might well be obtained with a transition state analog of the non-B_{12} enzyme.

The reaction mechanism of the B_{12}-containing enzyme has been extensively studied and is known to involve a methyl-B_{12} intermediate (Fig. 10). The methyl group is first transferred from the tetrahydrofolate derivative to vitamin B_{12}. The methyl-B_{12} compound then donates the methyl group to homocysteine to form methionine. The mechanism of the non-B_{12} enzyme has not been studied. To illustrate the usefulness of a transition state analog, however, a simple and attractive mechanism can be postulated (Fig. 11). It involves the "activation" of the methyl bearing nitrogen on the tetrahydrofolate derivative. Uncharged nitrogen has a pair of electrons not involved in bond formation, represented as dots. As previously discussed, the donation of these electrons to form a new bond (in this case to hydrogen ion, H^+) makes the nitrogen positively charged. The next step in the postulated mechanism is the "attack" on the methyl group by a pair of electrons from the sulfur, displacing the electrons from the methyl-nitrogen bond to the nitrogen. The result is the overall reaction shown in Fig. 10.

The transition state of this reaction is shown with half-formed bonds represented on dotted lines. An analog of this transition state is shown in Fig. 12. The sulfur-CH_2-nitrogen linkage mimics the transition state of the non-B_{12} catalyzed reaction. The reader should

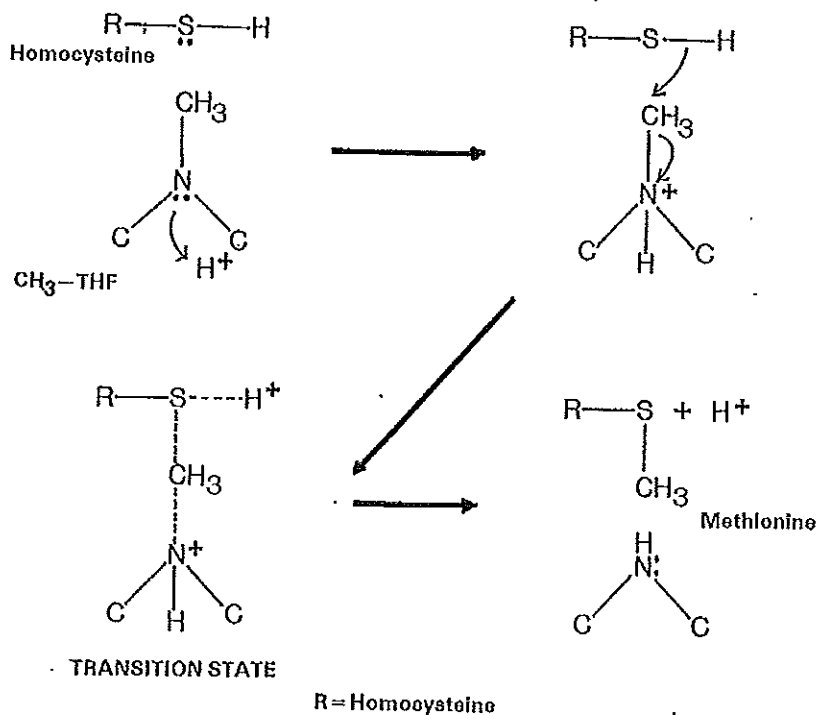

TRANSITION STATE

$R = $ Homocysteine

Fig. 11. An abbreviated diagram of the hypothesized mechanism of methyl transfer in the non-B_{12} enzyme. Notice that in the transition state the sulfur, CH_3, and nitrogen groups are lined up adjacent to each other. This never happens in the course of B_{12} enzyme catalyzed reaction.

note that at no point in the vitamin B_{12} mediated reaction would one ever expect the configuration of atoms that occurs at the transition state of Fig. 11—a configuration mimicked by the compound in Fig. 12. Thus, as a transition state analog for the non-B_{12} enzyme and not the B_{12} enzyme, the compound in Fig. 12 would be expected to be aselective inhibitor of the non-B_{12} enzyme.

There are many more examples from Coward's laboratory of the use of transition state analogs as enzyme inhibitors. Also, different laboratories have produced other inhibitors designed using this or other rational approaches based on enzyme catalytic mechanism. These approaches have often met with much success, enabling experimenters to manipulate enzymes both *in vivo* and *in vitro*. Because of such successes, the use of transition state analogs and other rational approaches for inhibitor design based on a knowledge of enzyme catalytic mechanism is rapidly becoming popu-
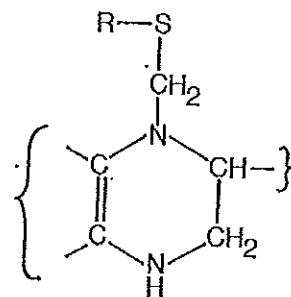

Fig. 12. Transition state analog for non-B_{12} enzyme.

lar as an alternative to random or semi-random screening processes for the development of enzyme inhibitors. As more biochemical information is gathered on the mechanism of disease, we should expect these rational approaches to be utilized effectively to design drugs for clinical use.