The Control of Biochemical Reactions

The cell is a factory and enzymes are its machines. Two feedback systems control production, one regulating synthesis of enzymes, another their activity. Models of the two systems are described

by Jean-Pierre Changeux

The analogy between a living organism and a machine holds true to a remarkable extent at all levels at which it is investigated. To be sure, living things are machines with exceptional powers, set apart from other machines by their ability to adapt to the environment and to reproduce themselves. Yet in all their functions they seem to obey mechanistic laws. An organism can be compared to an automatic factory. Its various structures work in unison, not independently; they respond quantitatively to given commands or stimuli; the system regulates itself by means of automatic controls consisting of specific feedback circuits.

These principles have long been recognized in the behavior of living organisms at the physiological level. In response to the tissues' need for more oxygen during exercise the heart speeds up its pumping of blood; in response to a rise in the blood-sugar level the pancreas increases its secretion of insulin. Now analogous systems have been discovered at work within the living cell. The new findings of molecular biology show that the cell is a mechanical microcosm: a chemical machine in which the various structures are interdependent and controlled by feedback systems quite similar to the systems devised by engineers who specialize in control theory. In this article we shall survey the experimental findings and hypotheses that have developed from the viewpoint that the cell is a self-regulating machine.

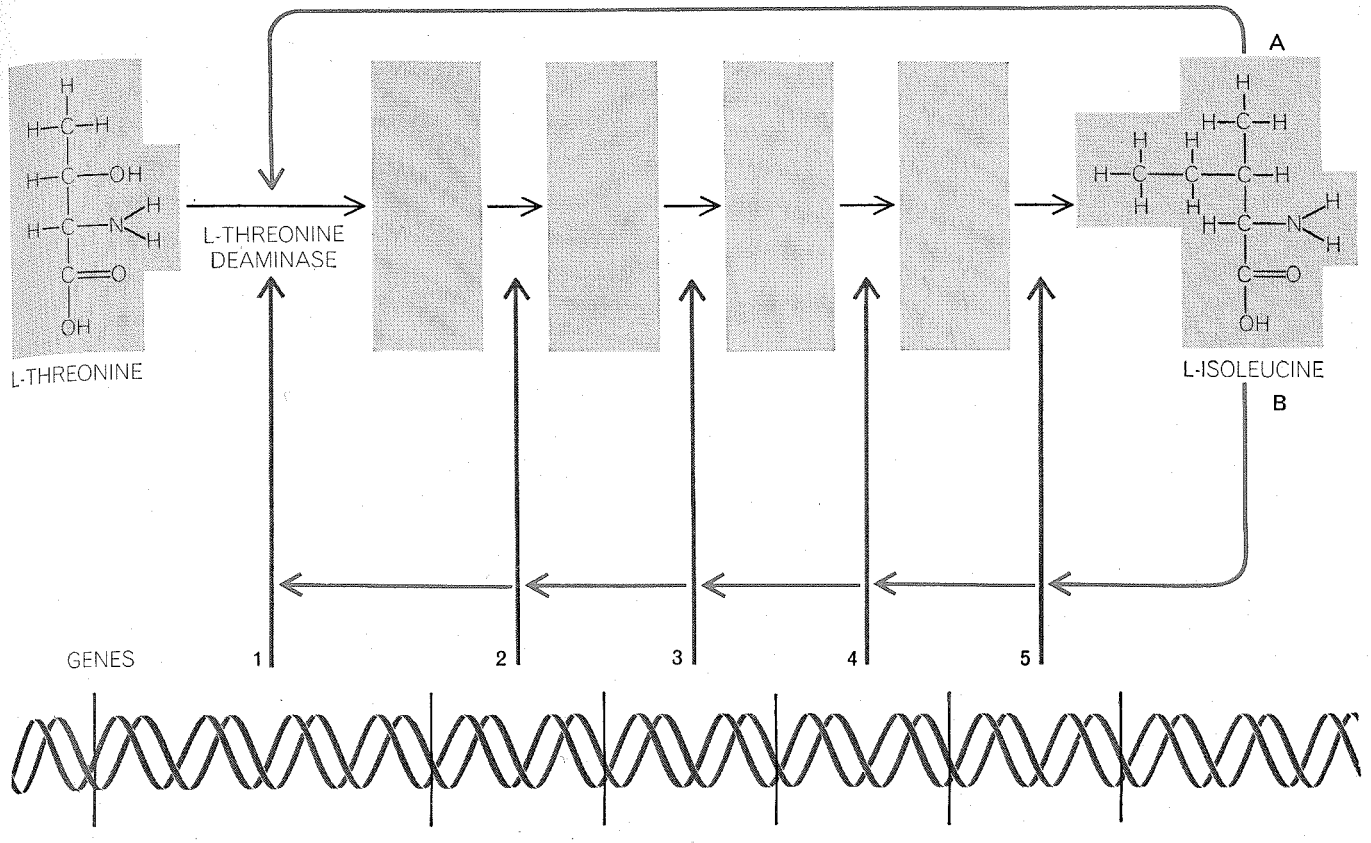
We can think of the cell as a completely automatic chemical factory designed to make the most economical use of the energy available to it. It manufactures certain products—for example proteins—by means of series of reactions that constitute its production lines, and most of the energy goes to power these processes. Regulating the production lines are control circuits that themselves require very little energy. Typically they consist of small, mobile molecules that act as "signals" and large molecules that act as "receptors" and translate the signals into biological activity.

The elementary machines of the cellular factory are the biological catalysts known as enzymes. The synthesis of any product (for example a specific protein) entails a series of steps, each of which calls for a specific enzyme. Obviously there are two possible ways in which the cell can control its output of a given product: (1) it may change the number of machines (enzyme molecules) available for some step in the chain or (2) it may change their rate of operation. Therefore in order to reduce the output of the product in question the cell may cut down the number of enzyme molecules or inhibit some of them or do both.

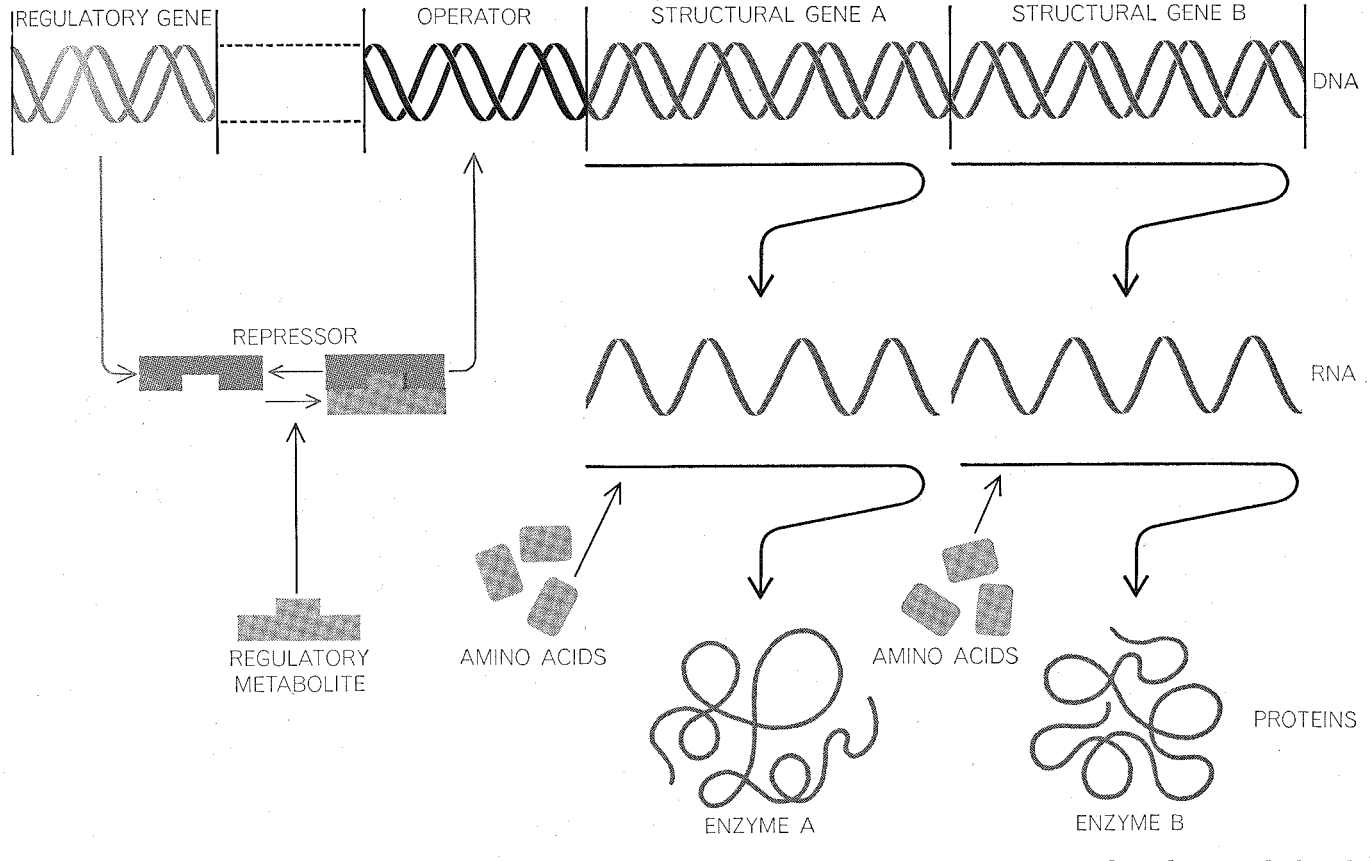
An excellent demonstration of such control has been obtained in experiments with the common bacterium *Escherichia coli*. The experiments involved the bacterial cell's production of the amino acid L-isoleucine, which it uses, along with other amino acids, to make proteins. Would the cell go on synthesizing this amino acid if it already had more than it needed for building proteins? L-isoleucine labeled with radioactive atoms was added to the medium in which the bacteria were growing; the experiments showed that when the substance was present in excess, the bacteria ceased to produce it. The amount of the amino acid in the cell in this case serves as the signal controlling its synthesis: if the amount is below a certain level, the cell produces more L-isoleucine; if it rises above that level, the cell stops producing L-isoleucine. Like the temperature level in a house with a thermostatically regulated heating system, the level of L-isoleucine in the cell exerts negative-feedback control on its own production.

How is the control carried out? H. Edwin Umbarger and his colleagues, working in the laboratory of the Long Island Biological Association, found that the presence of an excess of L-isoleucine has two effects on the cell: it inhibits the activity of the enzyme (L-threonine deaminase) needed for the first step in the chain of synthesizing reactions, and it stops production by the cell of all the enzymes (including L-threonine deaminase) required for L-isoleucine synthesis. Curiously it turned out that the two control mechanisms are independent of each other. By experiments with mutant strains of *E. coli* it was found that one mutation deprived the cell of the ability represented by the inhibition of L-threonine deaminase by L-isoleucine; another mutation deprived it of the ability to halt production of the entire set of enzymes. The two mutations were located at different places on the bacterial chromosome. Therefore it is clear that the two control mechanisms are completely separate.

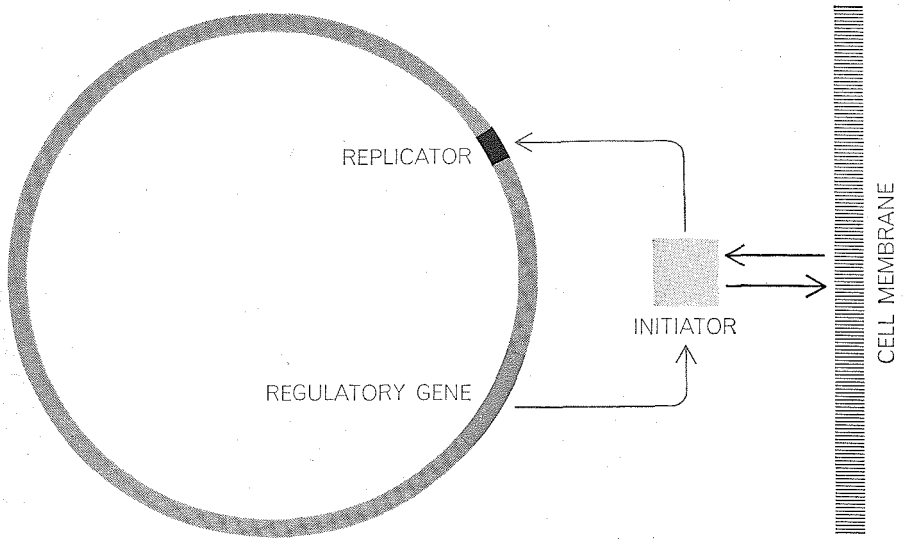
Let us first examine the type of mechanism that controls the manufacture of enzymes. It was Jacques Monod and Germaine Cohen-Bazire of the Pasteur Institute in Paris who discovered the phenomenon of repression: the inhibition of enzyme synthesis by the presence of the product, the product serving as a signal that the enzymes are not needed. The signal substance



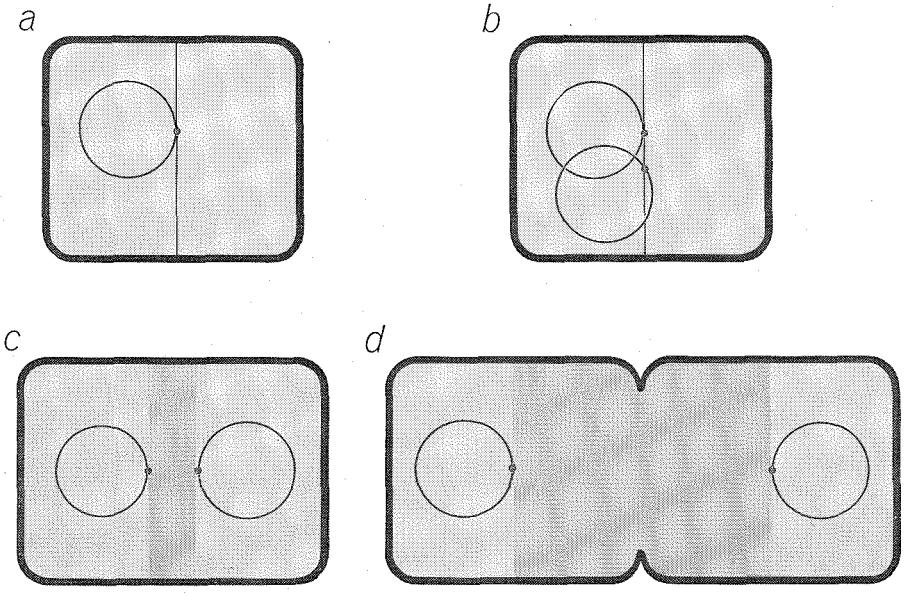
TWO FEEDBACK SYSTEMS control the biosynthesis of cell products, as shown here for the synthesis of the amino acid L-isoleucine in the bacterium *Escherichia coli*. The end product of the synthesizing chain acts as a regulatory signal that inhibits the activity of the first enzyme in the chain, L-threonine deaminase (*A*), and also represses the synthesis of all the enzymes (*B*).



CONTROL OF PROTEIN SYNTHESIS by a genetic "repressor" was proposed by François Jacob and Jacques Monod. A regulatory gene directs the synthesis of a molecule, the repressor, that binds a metabolite acting as a regulatory signal. This binding either activates or inactivates the repressor, depending on whether the system is "repressible" or "inducible." In its active state the repressor binds the genetic "operator," thereby causing it to switch off the structural genes that direct the synthesis of the enzymes.



REPLICATION OF DNA of a bacterial chromosome may be under a control like that of protein synthesis. A regulatory gene directs the synthesis of an "initiator," which receives a signal (perhaps from the cell membrane) that makes it act on the "replicator."



ROLE OF CELL MEMBRANE in replication is suggested by the fact that a bacterial chromosome is attached to a point on the membrane (*a*). It could be a signal from the membrane that initiates the formation of daughter chromosomes (*b*). Then the membrane begins to grow, separating the points of attachment (*c*) until the cell is ready to divide (*d*).

in their experiments was the amino acid tryptophan. They found that when the medium in which *E. coli* cells were growing contained an abundance of tryptophan, the cells stopped producing tryptophan synthetase, the enzyme required for the synthesis of the amino acid. This efficient behavior has since been demonstrated in many cells, not only bacteria but also the cells of higher organisms. The addition of an essential product to the cells' growth medium results in a negative-feedback signal that causes them to stop synthesizing enzymes they do not need.

In other systems the response of the cell is not negative but positive. We have been considering signals that repress the synthesis of enzymes; the cell can also respond to signals calling on it to produce enzymes. An example of such a situation is that the cell is confronted with a compound it must break down into substances it requires for growth.

The "induction" of enzyme synthesis in cells was discovered at the turn of the century by Frédéric Dienert of the Agronomical Institute in France. He was studying the effect of a yeast (*Saccharomyces ludwigii*) in fermenting the milk sugar lactose. He found that strains of the yeast that had been grown for several generations in a medium containing lactose would begin to work on the sugar immediately, causing it to start fermenting within an hour. These cells had a high level of lactase, an enzyme that specifically breaks down lactose. Yeast cells that had not been grown in lactose lacked this enzyme, and not surprisingly they failed to ferment lactose on being introduced to the sugar. After 14 hours, however, fermentation of the sugar did get under way; it developed that the presence of the lactose had induced the yeast to produce the enzyme lactase. The adaptation was quite specific: only lactose caused the yeast to synthesize this enzyme; other sugars failed to do so.

In recent years Monod and François Jacob of the Pasteur Institute have worked out some of the basic mechanisms of enzymatic adaptation by the cell, in both the repression and induction aspects. First they discovered that a single mutation in *E. coli* could eliminate the control of lactase synthesis by lactose: the mutant cells produced lactase just as well in the absence of lactose as in its presence. In these cells only the triggering effect was changed; the enzyme they produced was exactly the same as that synthesized by nonmutant strains. In other words, it appeared that the rate of production of the enzyme was controlled by one gene and that the structure of the enzyme was determined by quite another gene. This was confirmed by genetic experiments that showed that the "regulatory gene" and the "structural gene" were indeed in separate positions on the bacterial chromosome.

How does the regulatory gene work? Arthur B. Pardee, Jacob and Monod found that it causes the cell to produce a "repressor" molecule that controls the functioning of the structural gene. In the absence of lactose the repressor molecule prevents the structural gene from directing the synthesis of lactase molecules. The repressor does not act on the structural gene directly; it binds itself to a special structure that is closely linked on the chromosome with the structural gene for the enzyme and with several other genes involved in lactose metabolism. This special genetic structure is called an "operator." The binding of the repressor to the operator causes the latter to switch off the activity of the adjacent structural genes,

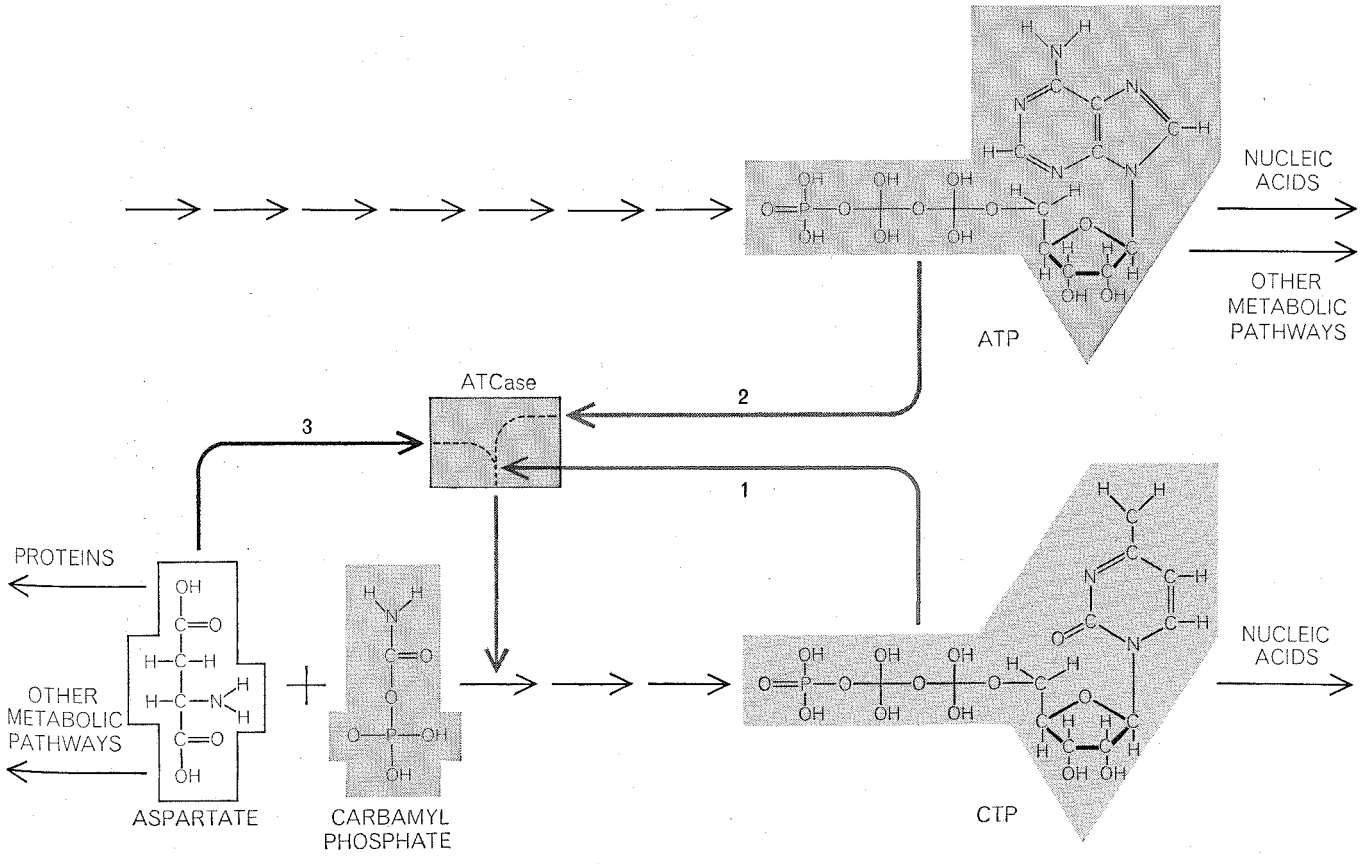
and in this way it blocks the complex series of events that would lead to synthesis of the enzyme.

Jacob and Monod have shown that this scheme of control applies to any category of "adaptive" enzymes [*see bottom illustration on page 37*]. The repression and induction of enzymes can be regarded as opposite sides of the same coin. In a repressible system the binding of the regulatory signal on the repressor activates the repressor so that it blocks the synthesis of the enzyme. In an inducible system, on the other hand, the binding of the inducing signal on the repressor *inactivates* the repressor, thus releasing the cell machinery to synthesize the enzyme. Mutant cells that lose the repressive machinery need no inducer: they synthesize the enzyme almost limitlessly without requiring any induction signal.

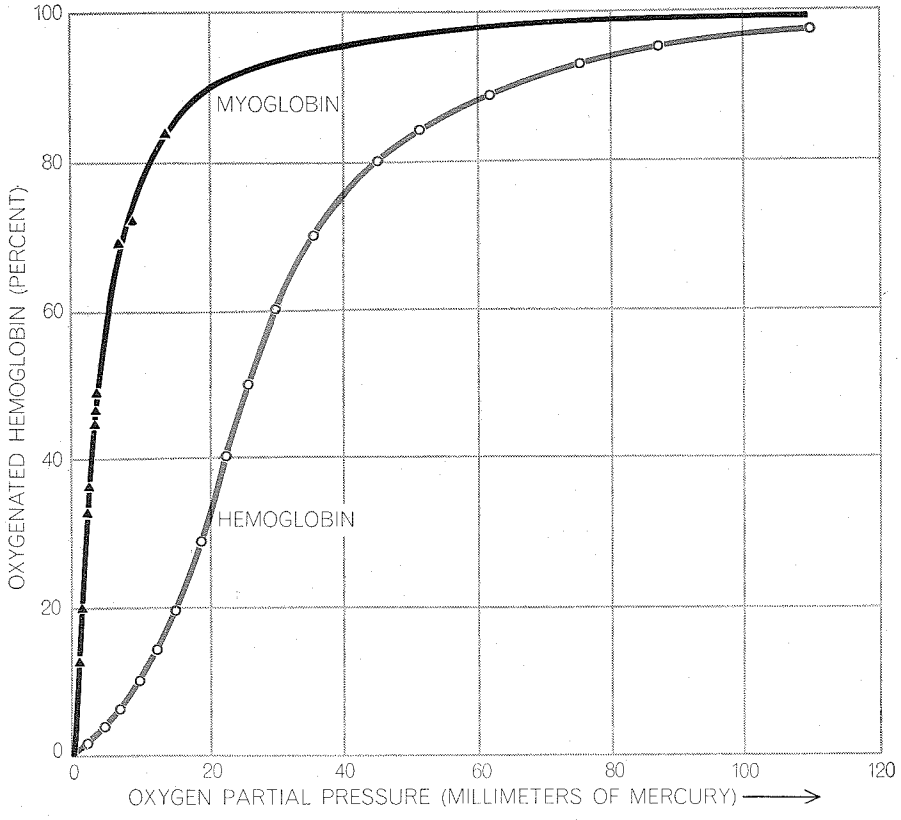
In brief, the various repressors in the cell are specialized receptors, each capable of recognizing a specific signal. And within its chromosomes a cell possesses instructions for synthesizing a wide variety of enzymes, each of which can be evoked simply by the presentation of the appropriate signal to the appropriate repressor.

The cell's selection of chromosomal records for transcription is so efficient as to seem almost "conscious." Actually, however, the responses of the cell are automatic, and like any other automatic mechanism they can be "tricked." It is as though a vending machine were made to work by a false coin: certain artificial compounds closely resembling lactose are excellent inducers of lactase but cannot be broken down by the enzyme. This means that the cell is tricked into spending energy to make an enzyme it cannot use. The signal works, but it is a false alarm. Trickery in the opposite direction is also possible. There is an analogue of tryptophan, called 5-methyl tryptophan, that acts as a repressive signal, causing the cell to stop its production of tryptophan. But 5-methyl tryptophan cannot be incorporated into protein in place of the genuine amino acid. Without that essential amino acid the cell stops growing and dies of starvation. Thus the false signal in effect acts as an antibiotic.

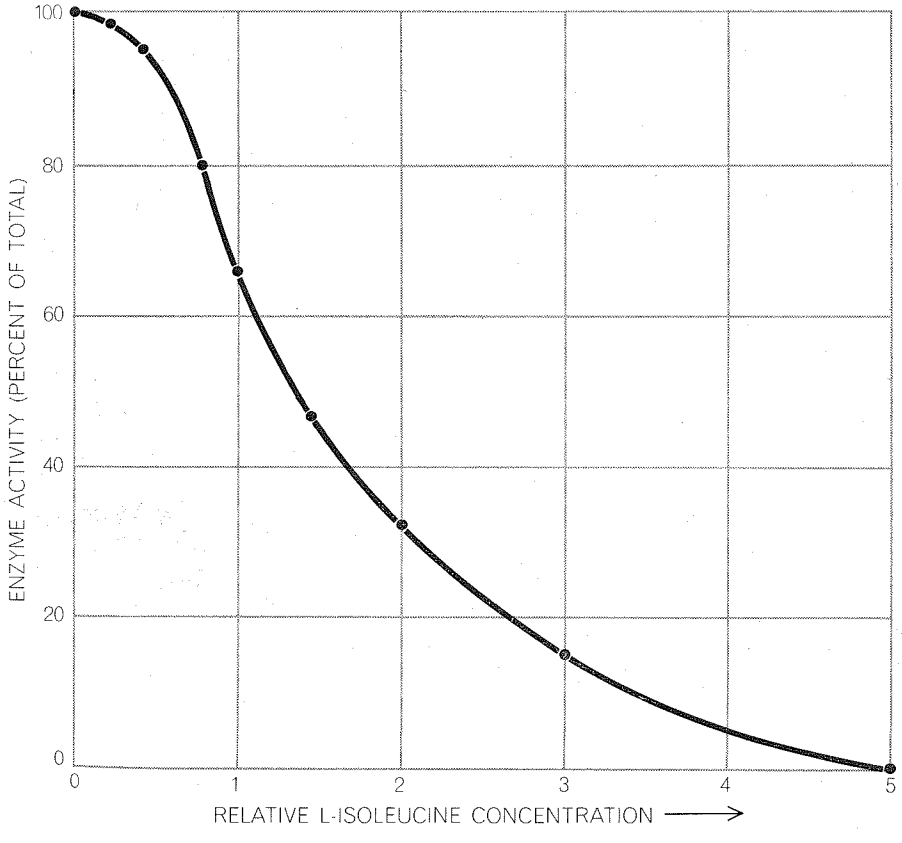
If chemical signals control the production of enzymes, may they not also control the more generalized activities of the cell, notably its self-replication? Jacob, Sydney Brenner and François Cuzin, working cooperatively at the Pasteur Institute and at the Laboratory of Molecular Biology at the University of Cambridge, recently discovered evidence of such a chemical control. They investigated the replication of the unique circular chromosome of *E. coli*. The synthesis of the deoxyribonucleic acid (DNA) of the chromosome, they found, is initiated by a signaling molecule that corresponds to the repressor of enzyme synthesis. The "initiator" has a positive effect rather than a repressive one. Like the repressor of enzyme synthesis, it is synthesized under the direction of a regulatory gene for replication. As the cell prepares for division, the initiator receives orders from the cell membrane and triggers the replication of its DNA by activating a genetic structure called the replicator (analogous to the "operator" of enzyme synthesis). Not much information has been gathered so far about the signal that prompts the initiator or about the



TWO NUCLEOTIDES, adenosine triphosphate (ATP) and cytidine triphosphate (CTP), are required by the cell in fixed proportions, so their production is regulated by interconnected feedback mechanisms operating on the first enzymes in the synthetic chains. In the case of CTP the enzyme is aspartate transcarbamylase (ATCase). It is inhibited by an excess of CTP (*1*), activated by an excess of ATP (*2*) and must also recognize and respond to the "cooperative" effects of aspartate, its substrate (*3*), which also plays a role in protein synthesis. Notice that ATP, CTP and aspartate have different shapes. How, then, can they all "fit" ATCase chemically?



HEMOGLOBIN, like an enzyme, is a large molecule that binds a small one (oxygen) at specific sites. The curves show the rate of oxygen-binding by hemoglobin (*color*) and myoglobin (*black*), a related oxygen-carrier in muscle. The myoglobin curve is a hyperbola but the hemoglobin curve is S-shaped. Hemoglobin binds best at higher oxygen concentrations (in the lungs); the binding of a few oxygen molecules favors the binding of more.



"COOPERATIVE EFFECT" occurs in regulatory enzymes as in hemoglobin. This curve shows the inhibition of L-threonine deaminase by L-isoleucine. The curve's *S* shape indicates that the effect of the regulatory signal is significant only above a threshold value.

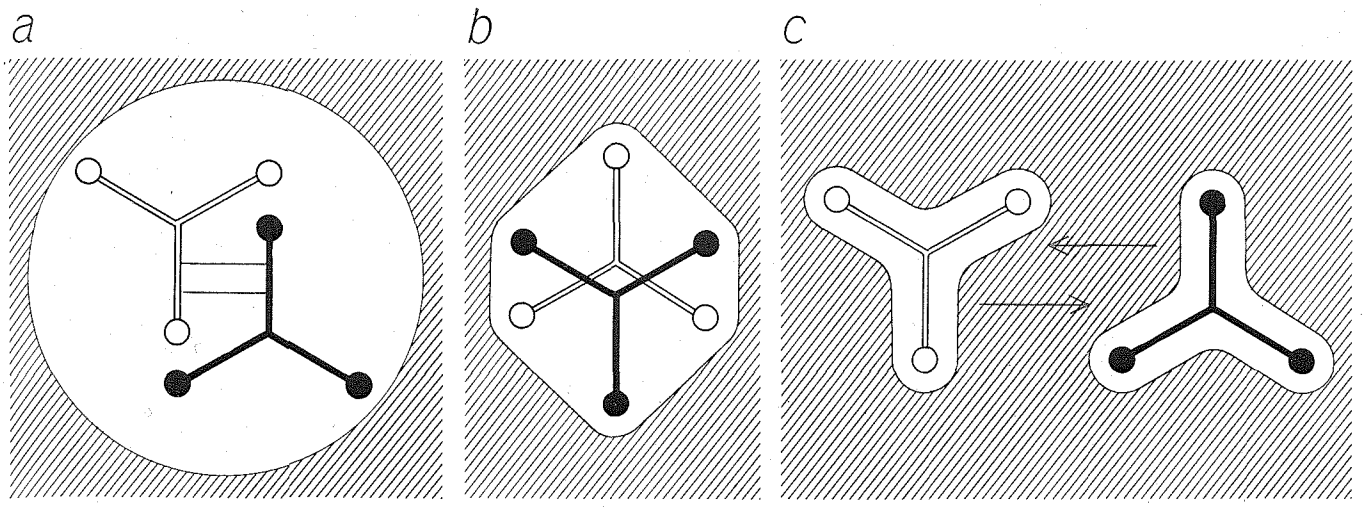
details of the machinery it sets in motion, but it seems clear that cell division has its own system of chemical control and that it can adjust itself to the composition of the growth medium.

We have been considering the control of the synthesis of enzymes; now let us turn to the control of their activity. As I have mentioned, Umbarger and his colleagues found that the presence of L-isoleucine would not only cause *E. coli* to stop synthesizing the enzymes needed for its production but also inhibit the activity of the first enzyme in the chain leading to the formation of the amino acid. The phenomenon of control of enzyme activity had already been noted earlier in the 1950's by Aaron Novick and Leo Szilard of the University of Chicago. They had shown that an excess of tryptophan in the *E. coli* cell halted the cell's production of tryptophan immediately, which means that the signal inhibited the activity of enzymes already present in the cell. Umbarger went on to investigate the direct effect of L-isoleucine on the enzymes that synthesize it; these had been extracted from the cell. He demonstrated that L-isoleucine inhibited the first enzyme in the chain (L-threonine deaminase), and only the first. This action was extremely specific; no other amino acid—not even D-isoleucine, the mirror image of L-isoleucine—had any effect on the enzyme's activity.

One must pause to remark on the extraordinary economy and efficiency of this control system. As soon as the supply of L-isoleucine reaches an adequate level, the cell stops making it at once. The signal acts simply by turning off the activity of the first enzyme; that is enough to stop the whole production line. Most remarkable of all, once this first enzyme has been synthesized the control costs the cell no expenditure of energy whatever; this is shown by the fact that the amino acid will act to inhibit the enzyme outside the cell without any energy being supplied. A factory with control relays that require no energy for their operation would be the ultimate in industrial efficiency!

The L-isoleucine control system of *E. coli* is only one example of this type of regulation in the living cell. It has now been demonstrated that similar circuits control the cell's production of the other amino acids, vitamins and other major substances, including the purine and pyrimidine bases that are the precursors of DNA.

In all these cases the control is nega-

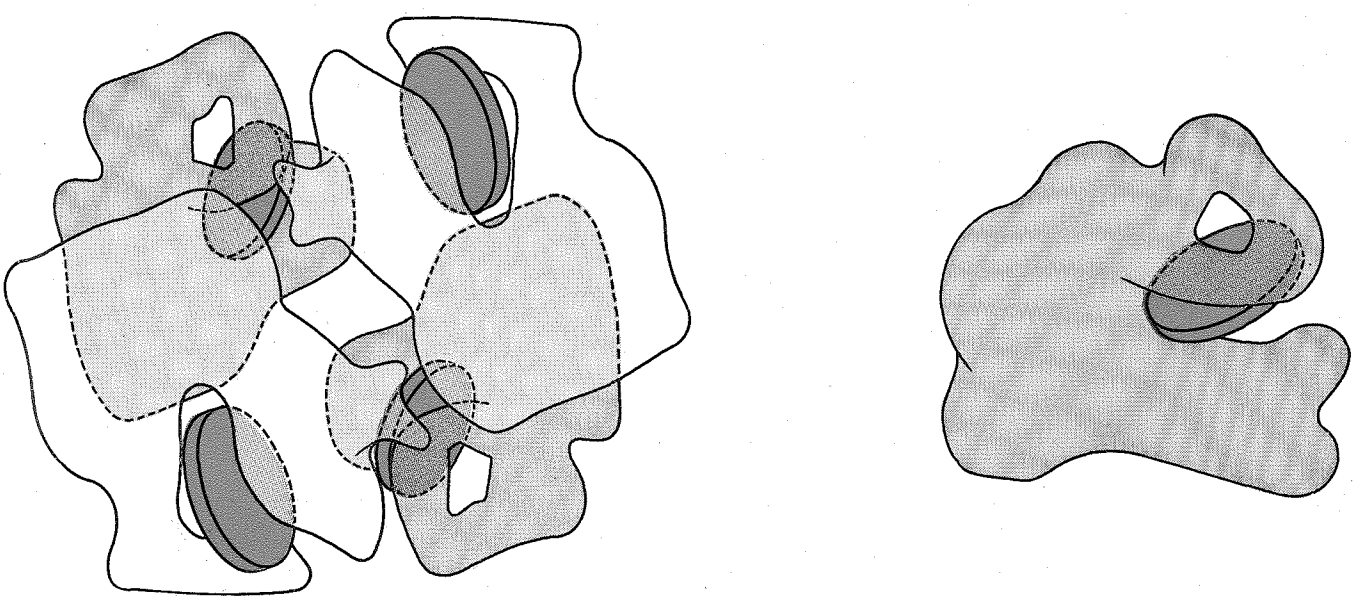


REGULATORY PROPERTY of an enzyme might be explained in three different ways. A regulatory signal (*open shape*) might combine with the substrate (*black shape*), participating directly in the chemical reaction it is controlling (*a*). But no such compounds have been found. A signal could simply get in the way of the substrate, excluding it from the enzyme's active site by "steric hindrance" (*b*). The different shapes of substrates and signals preclude this, and in any case steric hindrance could only account for enzyme inhibition, not activation. The only plausible hypothesis, confirmed by experiments with several enzymes, is that the signals and the substrate fit different sites on the enzyme and that the regulatory interactions of these sites are "allosteric," or indirect (*c*).

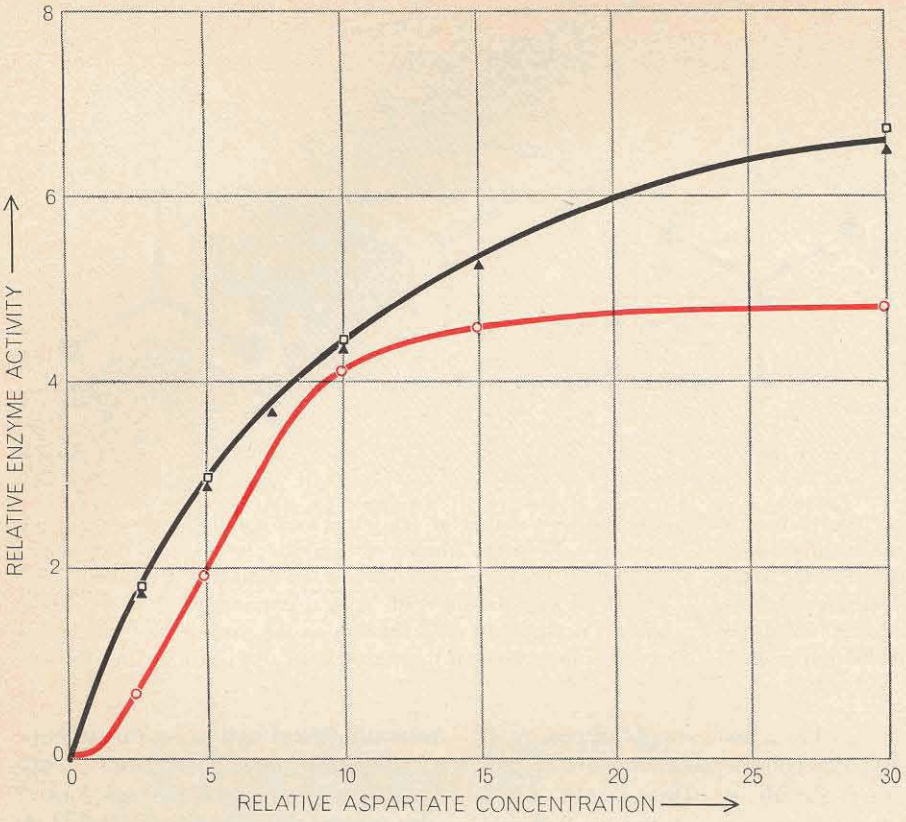
tive; that is, it involves the inhibition of enzymes. There are opposite situations, of course, in which the control system *activates* an enzyme when the circumstances call for it. An excellent example of such a positive control has to do with the cell's storage and use of energy.

Animal cells store reserve energy in the form of glycogen, or animal starch. Glycogen is synthesized from a precursor—glucose-6-phosphate—in three enzymatic steps. First glucose-6-phosphate is made into glucose-1-phosphate; then glucose-1-phosphate is made into uridine diphosphate D-glucose. Finally uridine diphosphate D-glucose is made into glycogen. When the cell has a good supply of energy, it produces considerable amounts of glucose-6-phosphate. This serves as a signal for stimulating the synthesis of glycogen. The signal works at the third step: the presence of a high level of glucose-6-phosphate strongly activates the enzyme that brings about the conversion of uridine diphosphate D-glucose into glycogen. On the other hand, when the supply of working energy in the cell falls to a low level, so that it must draw on the reserve stored in glycogen, it becomes necessary to activate an enzyme that splits the glycogen (the enzyme known as glycogen phosphorylase). One chemical signal known to be capable of activating this enzyme is adenosine monophosphate (AMP). AMP is a product of the splitting of adenosine triphosphate (ATP), the principal source of the cell's working energy, and an accumulation of AMP therefore indicates that the cell has used up its energy. The AMP signal activates the glycogen-splitting enzyme; the enzyme splits the glycogen molecule; the splitting releases energy, and the energy then is used to regenerate ATP.

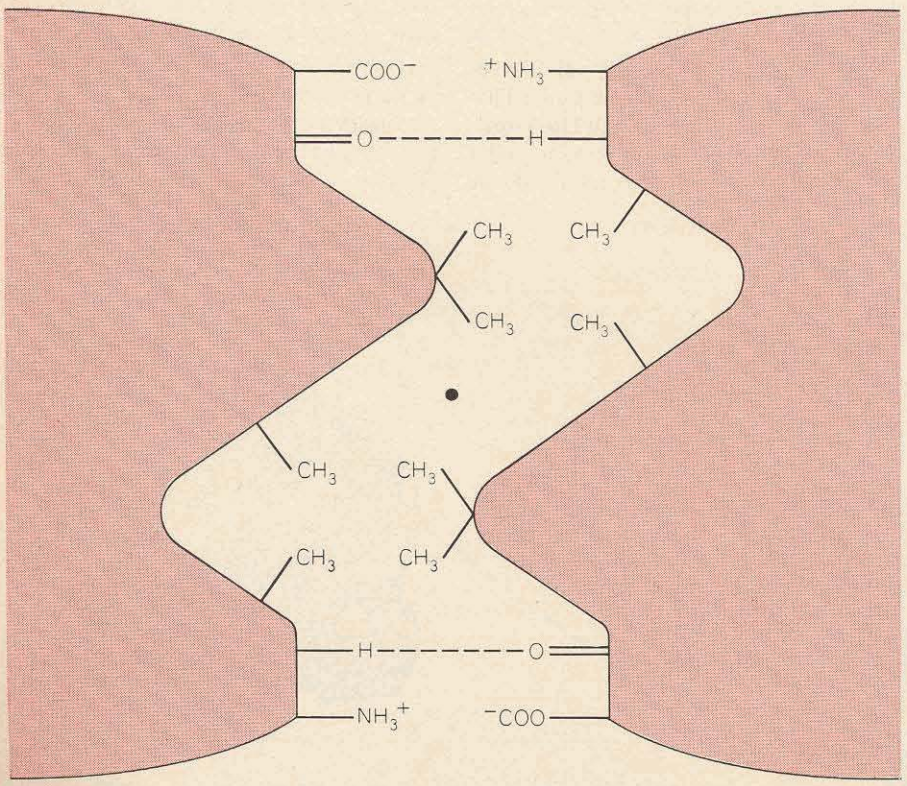
The cell thus possesses mechanisms for two types of control of enzyme activity: negative (inhibited enzymes) and positive (activated enzymes). There are



MOLECULE OF HEMOGLOBIN, shown (*left*) in very simplified form, has four heme groups (*color*), each of which is borne on a subunit, or chain, that is very similar to a myoglobin molecule (*right*). The heme groups of hemoglobin, each of which is a binding site for an oxygen molecule, are relatively far apart. Cooperative interactions among them must therefore be "allosteric."



DESENSITIZATION of an enzyme affects all its regulatory properties. The substrate saturation curve of natural ATCase (*color*) is *S*-shaped as a result of the cooperative effect. If the enzyme is denatured by heating, the cooperative effect is lost (*black curve*). So is the effect of feedback inhibition by CTP, as shown by the fact that the curve is the same whether the enzyme is assayed without CTP (*triangles*) or with CTP added (*squares*).



ALLOSTERIC PROTEINS are assumed by Monod, Jeffries Wyman and the author to be polymers, molecules composed of identical subunits, that have a definite axis of symmetry (*black dot*). A cross section through such a molecule (made up in this case of two subunits) shows how the symmetry results from the chemical bonds by which the units are associated.

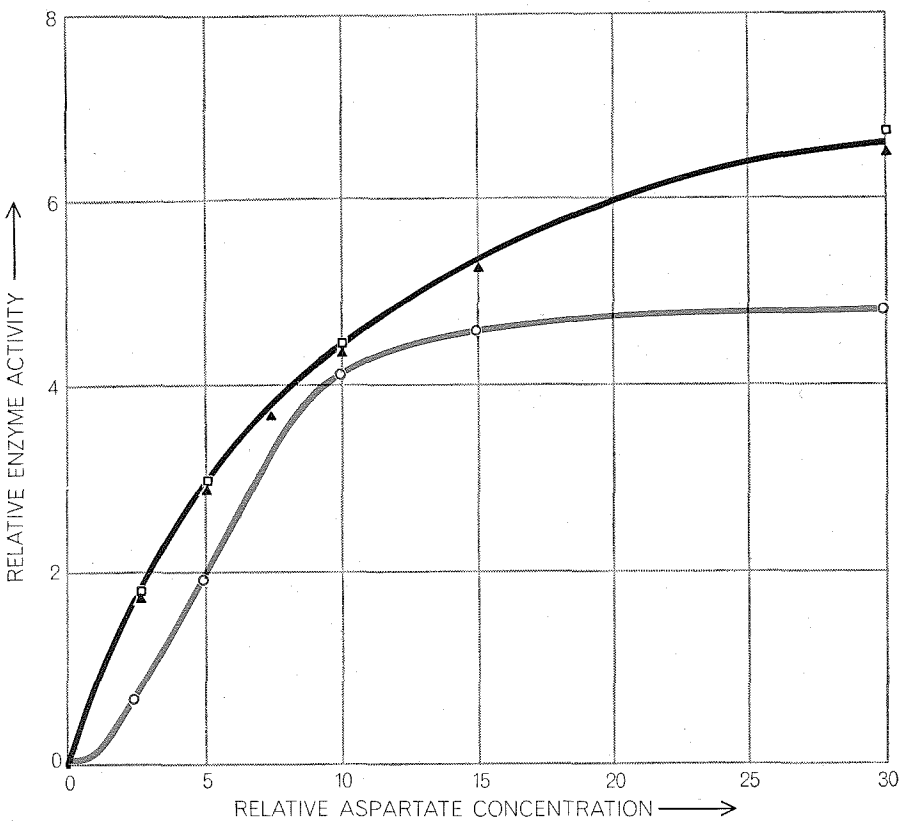
situations in which both methods operate simultaneously. Consider, for example, the synthesis of a nucleic acid. It is assembled from purine and pyrimidine bases, combined in certain definite proportions. The purines and pyrimidines are synthesized on parallel production lines. For the sake of economy they should be produced roughly in the proportions in which they will be used.

This implies that the rate of production by each production line should feed back to control the output by the other. Such a system of mutual regulation must employ both negative and positive controls. Exactly this kind of system has been demonstrated in experiments with *E. coli* conducted by John C. Gerhart and Pardee at the University of California at Berkeley and at Princeton University. They showed that the output of the pyrimidine production line is controlled not only by its own end product (which inhibits the first enzyme in the synthetic sequence) but also by the end product of the purine production line, which counteracts the inhibition by the pyrimidine end product in vitro. Indeed, the purine end product can activate the pyrimidine production directly when no pyrimidine product is present! In short, the enzyme involved here is inhibited by one signal and activated by another.

Several enzymes involved in regulation have also been found to respond in this way to different signals. Moreover, this is not the only exceptional property of these enzymes. Let us now consider another property that will clarify the mechanism by which they are controlled.

A clue to this property seems to lie in the shape of the curve describing the rate at which the enzymes react with their substrates: the substances whose changes they catalyze. Ordinarily the rate of reaction of an enzyme increases as the concentration of substrate is increased. The increase is described by an experimental curve that fits a hyperbola. This kind of curve expresses the fact that the first step in the transformation of the substrate by the enzyme is the binding of the substrate to a specific attachment site on the enzyme.

When the concentration of substrate is increased, molecules of substrate tend to occupy more and more binding sites. Since the number of enzyme molecules is limited, at high concentrations of substrate nearly all the binding sites are occupied. At this point the rate of reaction levels off, hence the hyperbolic



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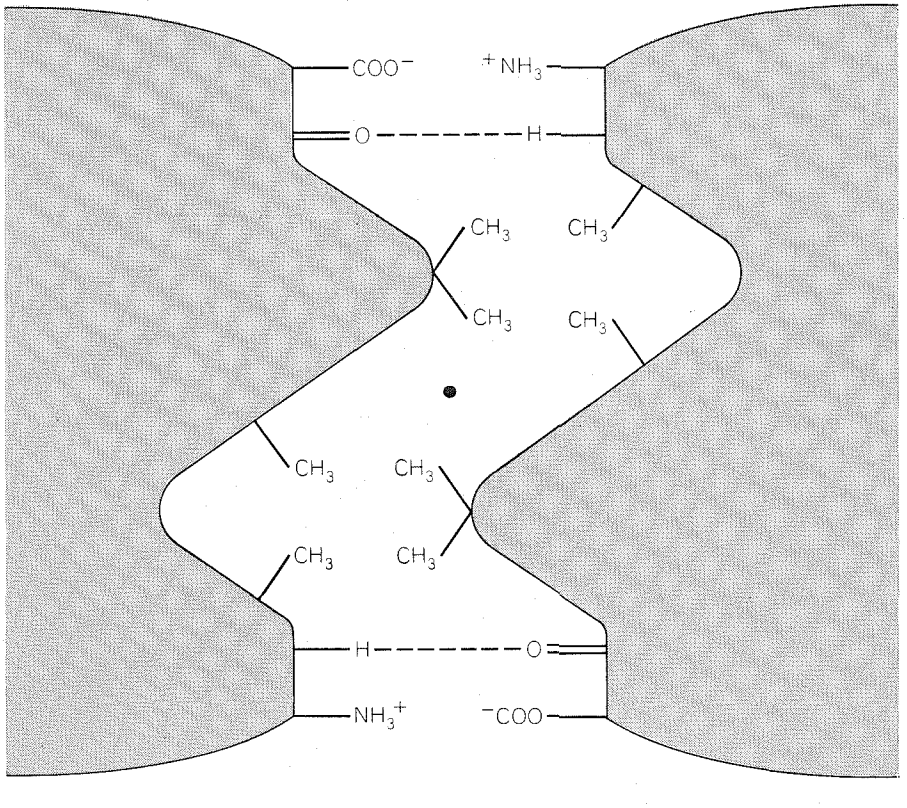

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shape of the curve. The regulatory enzymes, surprisingly, do not exactly follow this pattern: their reaction rate increases with the concentration of substrate but often the curve is sigmoid (S-shaped) rather than hyperbolic.

When one reflects on the saturation curve of the regulatory enzymes, one notes that it is strikingly like the curve describing the saturation of the hemoglobin of the blood with oxygen. There too the reaction rate traces a sigmoid curve; this remarkable property is related to hemoglobin's physiological function of carrying oxygen from the lungs to other tissues. In the lungs, where the oxygen pressure is high, the hemoglobin is readily charged with the gas; in the tissues, where the oxygen pressure is low, the hemoglobin readily discharges its oxygen. Consider now, however, the myoglobin of muscle tissue. It takes on oxygen, but its oxygenation follows a hyperbolic curve like the classical one for enzymes. A comparative chart shows that when the pressure of oxygen is increased, the amount of oxygen bound by hemoglobin increases faster than the amount bound by myoglobin [*see top illustration on page 40*]. It looks as if the first oxygen molecules picked up by the hemoglobin favor the binding of others—as if there is cooperation among the oxygen molecules in binding themselves to the carrier. Oxygen thus plays the role of a regulatory signal for its own binding.

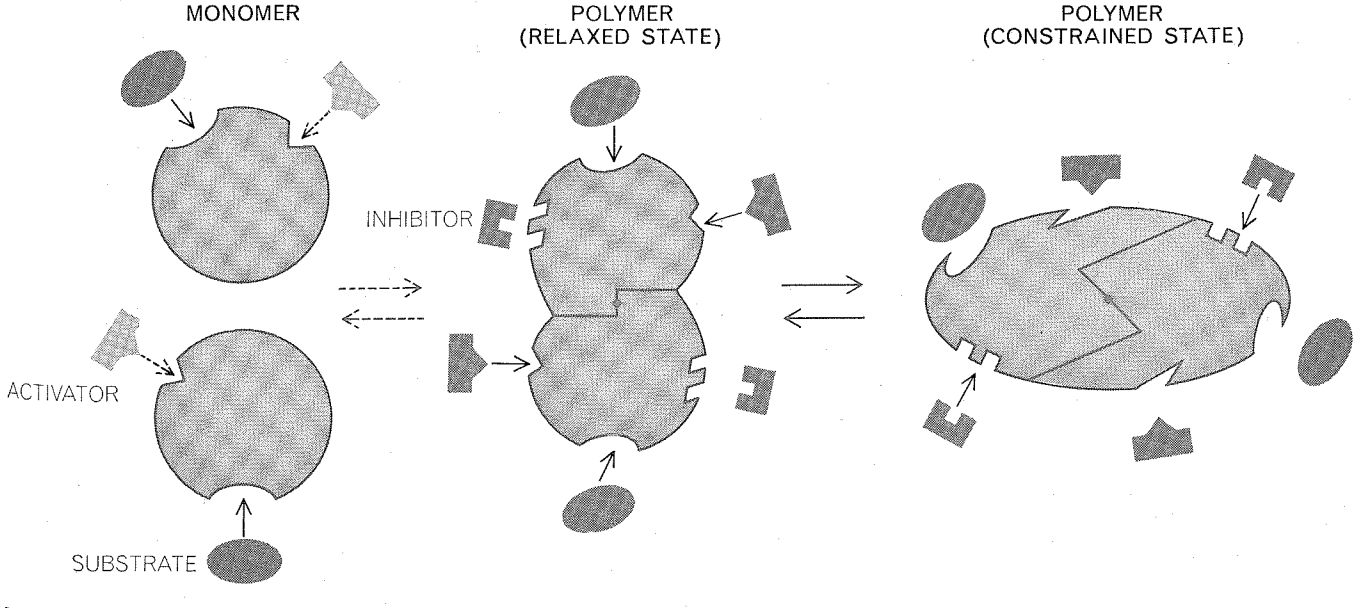
Similarly, cooperation may be the key to the sigmoid pattern of binding activity in many of the regulatory enzymes. An example of such an enzyme is threonine deaminase. Here again physiological function is evident. The substrate of threonine deaminase is the amino acid threonine. If the amount of this amino acid falls to a very low level in the cell, the cell cannot synthesize proteins. In the absence of threonine, it would be a waste of energy to make isoleucine, the end product of the chain of which threonine deaminase is the first step; hence the economy-geared control system of the cell calls off the production of the second amino acid. In other words, threonine deaminase will not be active and isoleucine will not be produced unless at least threshold concentrations of threonine are present in the cell. In this situation threonine plays the role of regulatory signal for the reaction of which it is the specific substrate; it is an activator of its own transformation.

The most remarkable part of the story is that such cooperative effects are not restricted to the binding of substrate but also operate in the binding of more familiar regulatory signals: specific inhibitors or activators. Regulatory enzymes appear to be built in such a way that they not only recognize the configuration of specific substrates as signals but also gauge their response to whether or not the substrates and regulatory signals are present in certain threshold concentrations. (This is strongly reminiscent, of course, of electric relays—and, one may add, of nerve cells—which react only if the signal has a certain threshold strength.) The regulatory enzymes are thus capable of integrating several signals—both positive and negative—that modulate their activity.

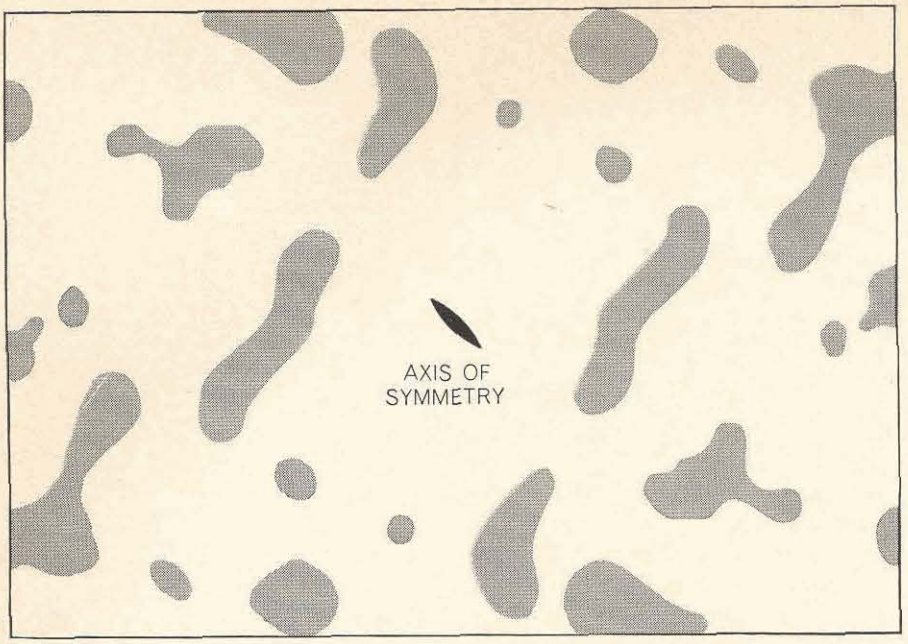
We come now to the question: How do the regulatory relays work? The signals (either activators or inhibitors) are usually small molecules, and the receptor is a regulatory enzyme. In chemical terms, how does the enzyme translate and integrate the signals it receives? The answer to this question applies not only to regulatory enzymes but also to any other molecule that mediates a regulatory interaction. Since little is known about many of these molecules, the model I shall now describe is based on the experimental results obtained from regulatory enzymes. It seems legitimate, however, to extend the model to any category of regulatory molecule.

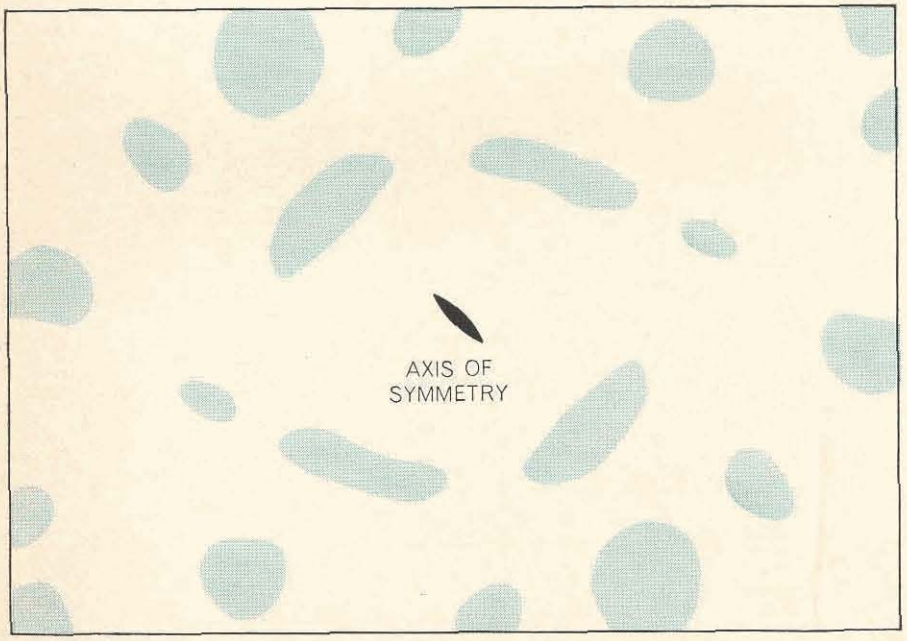
The question presents a biochemist with a difficult paradox. A molecule can "recognize" a message only in terms of geometry, that is, the shape or configuration of the molecule bearing the message. In this case the message is supposed to cause the enzyme to carry out (or refrain from carrying out) a certain reaction: conversion of a specific substrate into a specific product. Yet the molecule bearing the message often has no structural likeness to either the substrate or the product! How, then, can it promote or interfere with the enzyme's performance of its specific catalytic action on this substrate?

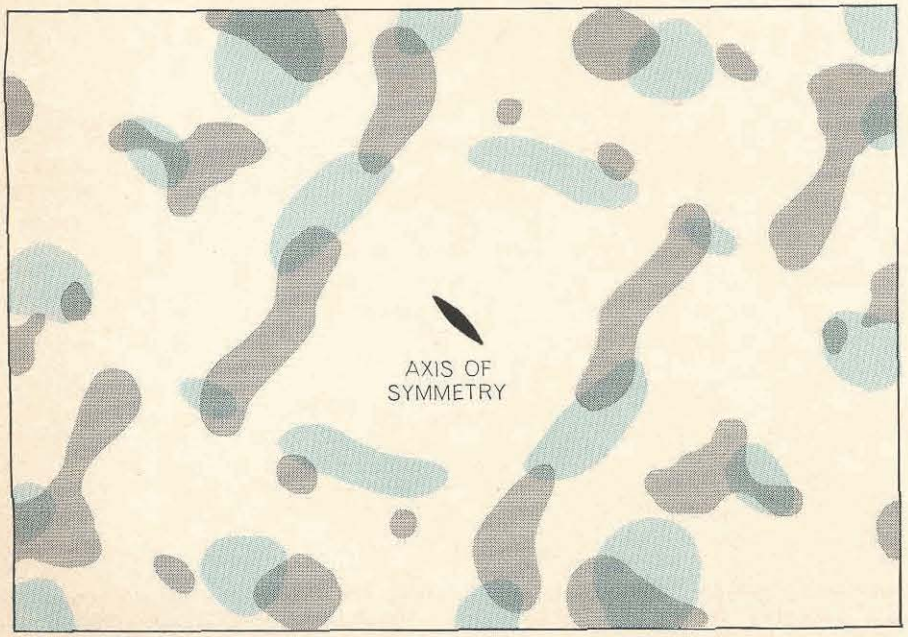
Considering several possible explana-



REGULATORY CHANGES in an allosteric molecule are conceived of as arising from its shifting back and forth between two states. The polymeric molecule is made up of several monomers (two in this case), as shown at left. The polymer can exist in a "relaxed" state (*middle*) or a "constrained" state (*right*). In one condition it binds substrate and activators; in the other state it binds inhibitors. The binding of a signal tilts the balance toward one or the other state but the molecule's symmetry is preserved.







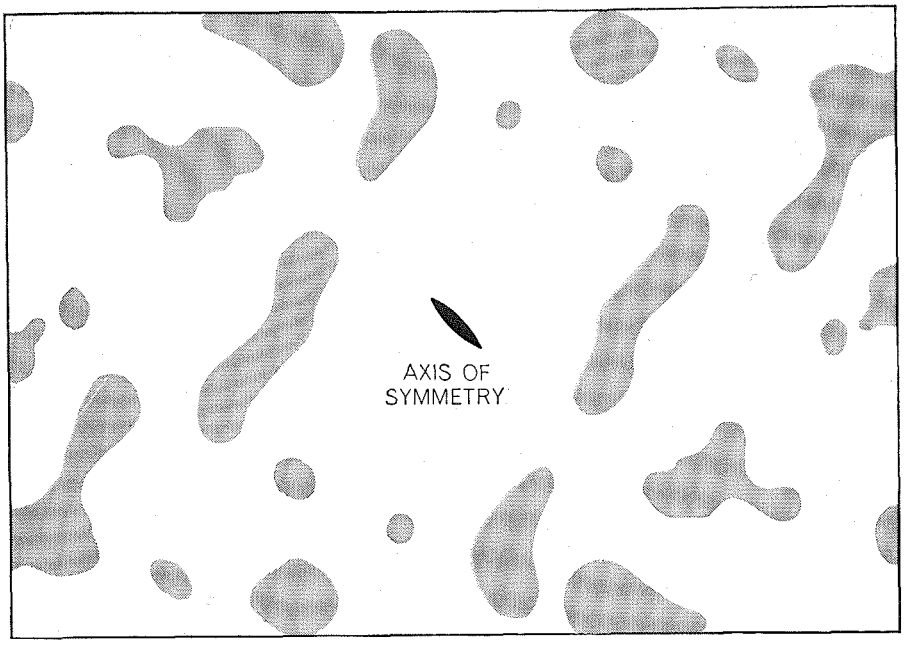
tions, Monod, Jacob and I have concluded that the only plausible one is that the signal and the substrate fit into separate binding sites on the enzyme and that the signal takes effect by an interaction between these sites [*see top illustration on page 41*]. There is strong experimental evidence in favor of this model. One of the most convincing lines of evidence is the recent discovery by Gerhart that the regulatory enzyme aspartate transcarbamylase has a binding site for its substrate on one subunit of the molecule and a site for an inhibitor of its activity on another subunit. When the subunits are split apart, one retains the ability to recognize the substrate, the other the ability to recognize the inhibitor.

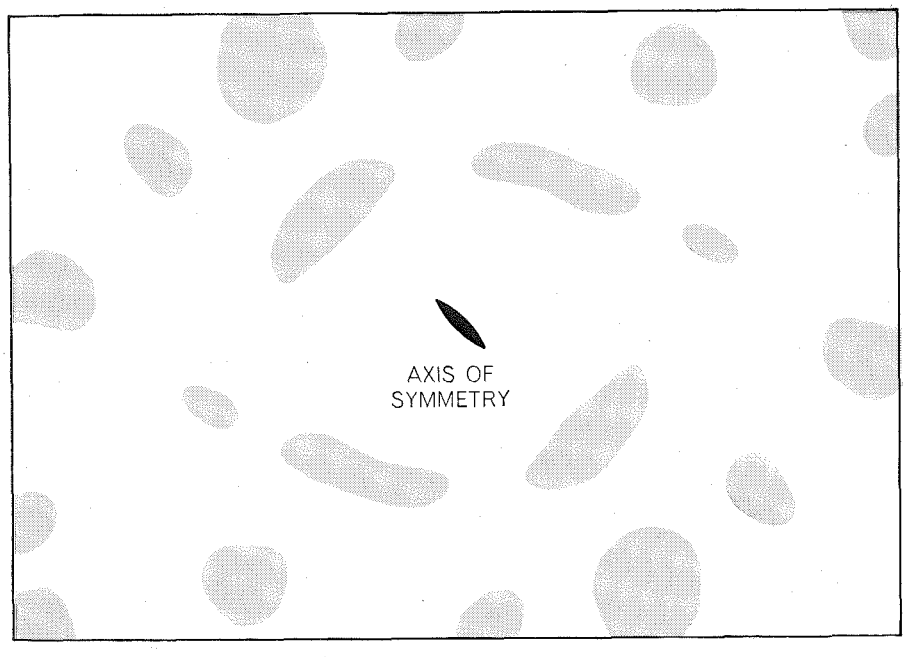
We must now inquire into the nature of the interaction of these two categories of sites on the enzyme. How does the binding of a molecule at one site affect the binding of another molecule at the other site? The best clue to an understanding of the mechanism of the interaction seems to lie in a property of regulatory enzymes that I have already mentioned: the sigmoid curve describing their binding of substrate or of signal molecules, which indicates a cooperative effect among those molecules. Again it is instructive to consider the analogy of the binding of oxygen molecules by hemoglobin.

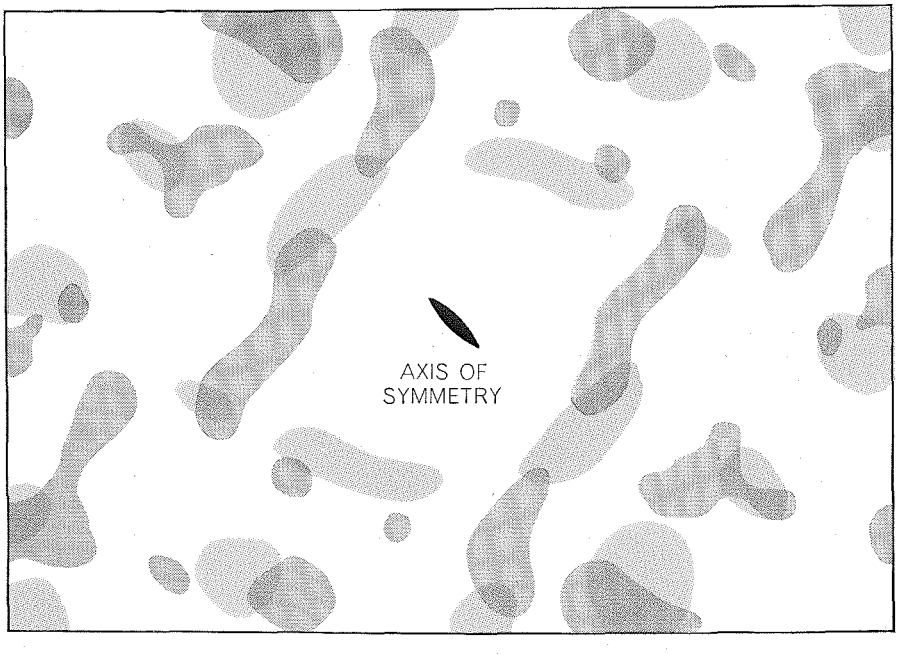
The hemoglobin molecule has four hemes that are well separated from one another; each is a binding site for an oxygen molecule. In view of the separation between the sites, their cooperation in binding oxygen must be "allosteric," or indirect. Myoglobin, which has only one binding site, binds oxygen hyperbolically (that is, without any control); hemoglobin, with its four sites, binds oxygen in a sigmoid pattern. It seems, therefore, that the key to hemoglobin's cooperative, controlled binding of oxygen lies in the molecule's four-part structure.

Now consider a regulatory enzyme. The binding of any particular molecule

EXPERIMENTAL DATA supporting the allosteric model come from X-ray diffraction maps of hemoglobin made by M. F. Perutz and his colleagues at the University of Cambridge. The contour lines based on electron densities suggest the shapes of the subunit chains of oxygenated hemoglobin (*top*), reduced hemoglobin (*middle*) and the two superposed (*bottom*). A conformational change of the kind proposed in the model on the preceding page is evident, as is preservation of the molecule's axis of symmetry.







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(substrate, inhibitor or activator) is sigmoid and therefore a cooperative affair; this implies that there is a set of reception sites for each specific molecule. There also appears to be interaction among the binding sites for different molecules, such as substrate and activator or substrate and inhibitor. Surprisingly the experimental evidence suggests that both types of allosteric interaction—that among the sites binding a particular molecule and that among the sites binding different molecules—may depend on one and the same mechanism, embodied in the structure of the enzyme molecule.

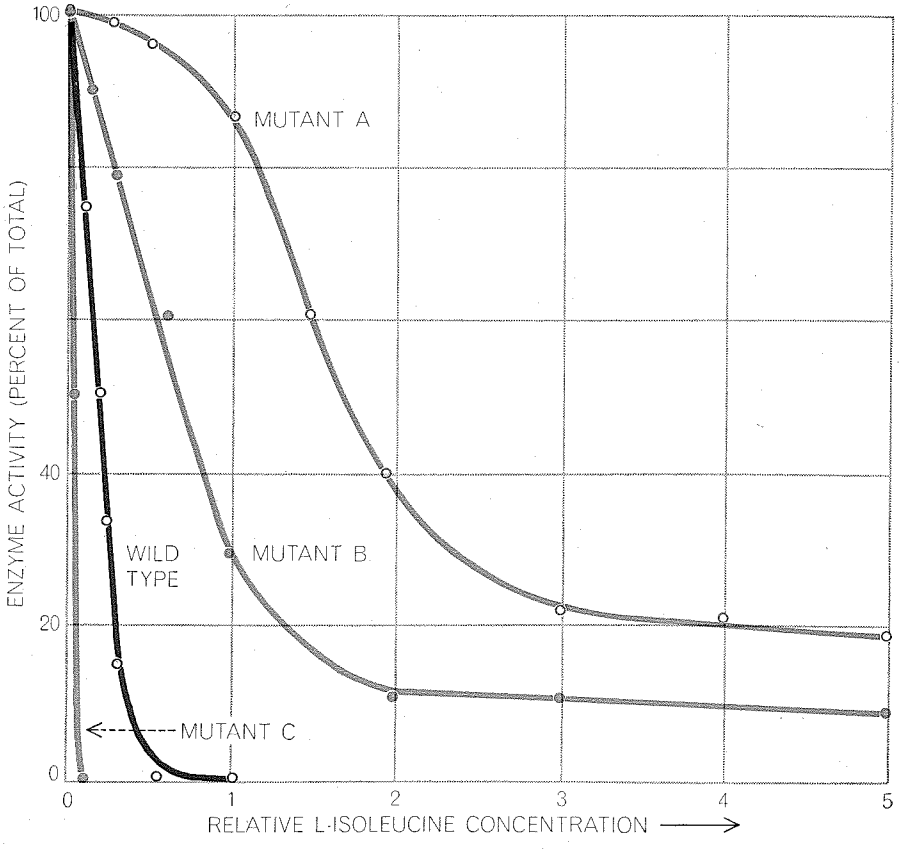
The most striking evidence comes from experiments in the alteration of the structure of regulatory enzyme molecules. Gerhart and Pardee at Berkeley and Princeton and I at the Pasteur Institute, working independently, have found that by changing the molecular structure of aspartate transcarbamylase or L-threonine deaminase (by means of heat, bacterial mutation or certain other procedures) it is possible to "desensitize" these regulatory enzymes so that they are no longer affected by a feedback inhibitor. They are still capable, however, of reacting with their respective substrates. The interesting point is that a change in the enzyme's structure eliminates, along with the negative interaction of the feedback inhibitor and the substrate, all the cooperative interactions in the enzyme molecule. This applies particularly to the binding of the substrate, which changes from a sigmoid to a hyperbolic pattern.

What, then, is the crucial structural feature that accounts for the allosteric interactions within the enzyme molecule? Again hemoglobin offers a clue.

We have noted that the hemoglobin molecule is a four-part structure. It comprises four heme units, each of which is attached to a distinct chain of amino acid units. This molecule is thus made up of four subunits, each of which is so similar to a myoglobin molecule that hemoglobin can be considered essentially a combination of four myoglobin molecules. Hemoglobin displays cooperative interaction, whereas myoglobin does not; hence this property evidently is associated with its four-part structure. Now, experiments show that the binding of oxygen by hemoglobin is connected in some way with an adjustment in the bonding between the subunits making up the molecule [see "The Hemoglobin Molecule," by M. F. Perutz; SCIENTIFIC AMERICAN, November, 1964]. The same turns out to be true of many of the regulatory enzymes; their binding of smaller molecules also depends on the adjustment of the bonds holding together their subunits.

On the strength of the experimental findings, Monod, Jeffries Wyman and I have proposed a model picturing the working of the regulatory enzyme system [*see illustration on page 43*]. It suggests that the enzyme molecule consists of a set of identical subunits, each subunit containing just one specific site for each of the molecules it may bind to itself, either substrate molecules or regulatory signals. Now, if a molecule is made up of a definite and limited number of subunits, the implication is that it has an axis of symmetry. Let us say that the enzyme molecule can switch back and forth between two states, and that in each state its symmetry is preserved. The two symmetrical states differ in the energy of bonding between the subunits: in the more relaxed state the enzyme molecule will preferentially bind activator and substrate; in the more constrained state it will bind inhibitor. Whichever compound it binds (substrate, inhibitor or activator) will tip the balance so that it then favors the binding of that category of small molecule. A change in the relative concentrations of substrate and signals may, depending on their molecular structure, tip the balance one way or the other. Thus the model indicates how the enzyme molecule's binding sites may interact, either cooperatively or antagonistically. It suggests that the enzyme may integrate different messages simply by adopting a characteristic state of spontaneous equilibrium between two states.

The major conclusion from the study of the regulatory enzymes is that their powers of control and regulation depend entirely on the form of their molecular structure. Built into that structure, as into a computer, is the capacity to recognize and integrate various signals. The enzyme molecule responds to the signals automatically with structural modifications that will determine the rate of production of the product in question. How did these biological "computers" come into being? Obviously they must owe their remarkable properties to nature's game of genetic mutation and selection, which in eons of time has refined their construction to a peak of exquisite efficiency.



MUTATIONS in the structural gene for L-threonine deaminase in *E. coli* affect the regulatory properties of the enzyme. Mutant enzymes respond differently to feedback inhibition.