cessity to provide bigger and more expensive special equipment if work is to be undertaken at all, it becomes increasingly impossible to provide such equipment at all the universities of the country. It seems likely that the day when every university can aspire to be at the forefront of all the fields of modern science is nearly at an end. I think, therefore, we will see the development of a variety of cooperative arrangements to cope with this problem. In high-energy physics this problem has been attacked through the concept of user groups by which national facilities are available to groups all over the country who carry out the analysis of records and their interpretation at their home sites but either perform experiments at the national centers or receive the raw material from experiments there for work. More generally, I suspect that additional associations of universities which are geographically proximate will be formed in which each can contribute to the strength of the other by maintaining special facilities which are available to all of the associating universities. Several such groups are now being formed, and the process is certainly a good one.

One problem to which I must make reference, but for which I cannot make predictions, is that everywhere there is concern with the plight of the small college and its role in the future. The problems are clear: the small colleges have difficulties recruiting faculties in the sciences; they have difficulties in providing the kind of facilities and the awareness of current change in science which is important if they are to continue to play the strong role they have in the past. There are some indications that their role is declining. I am sure that increased federal attention will be placed in this area through programs in various agencies, but I cannot now predict their form.

I have mentioned earlier the trend toward more general-purpose support. Examples of such programs in science might include the traineeship programs of the NIH and NSF, the various facilities programs and equipment programs, the general research support grants of the NIH for medical schools, and the institutional grants of the NSF. What is new is the assistance being provided to universities in all fields of study by the Office of Education as a result of several recent acts. These include facilities, fellowships, scholarships, loan funds, assistance for libraries, and so on. It seems very likely to me that there will be more.

In closing I want to return to my original theme. The close association of the federal government and the universities in performing many public functions is here to stay. The successful experience we have had so far gives me the greatest confidence that we will continue to develop that association in a way which responds to the needs of all parts of our country and all segments of our population, which places greater responsibility on the university as an institution to plot its course and determine its destiny, and which preserves the freedom of the individual scientist to pursue understanding according to his own insights.

Biological Feedback Control at the Molecular Level

Interaction between metabolite-modulated enzymes seems to be a major factor in metabolic regulation.

Daniel E. Atkinson

A living cell consists in large part of a concentrated mixture of hundreds of different enzymes, each a highly effective catalyst for one or more chemical reactions involving other components of the cell. The paradox of intense and highly diverse chemical activity on the one hand and strongly poised chemical stability (biological homeostasis) on the other is one of the most challenging problems of biology.

The first clear demonstrations of metabolic regulation at the molecular level by mechanisms other than mass action came in connection with biosynthetic sequences. This area has been adequately reviewed (*1*). In a short paper that already may be considered a classic, Umbarger (*2*) supplied the conceptual foundation for the operation of regulatory controls through specific interaction of the synthetic product with an enzyme early in its synthesis and also furnished an experimental demonstration of such control by showing that in extracts of disrupted cells, isoleucine strongly and specifically inhibits threonine dehydrase, the first enzyme unique to its synthesis. He pointed out the apparent advantages for the organism of such regulation at the molecular level and suggested a comparison with technological negative feedback-control devices. Another example of the same type of regulation was recognized at about the same time by Yates and Pardee (*3*), who discovered product feedback control in the biosynthesis of pyrimidines.

Enzyme-Effector Interaction

Although they are often used, in this article and elsewhere, for reasons of convenience, the terms "stimulation" and "inhibition" do not adequately describe the action of the regulatory metabolite (termed effector, modifier, or modulator) on the enzyme. The effector typically modifies the affinity of the enzyme for its substrates and frequently also for other reaction components. The terms positive and nega-

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tive effector may be used to indicate whether presence of the effector leads to increased or decreased affinity of the enzyme for substrates. Different regulatory enzymes are of course not identical in kinetic pattern, but the pattern shown in Fig. 1 may be taken as representative. The plot of reaction rate against substrate concentration for these enzymes is typically sigmoid, or S-shaped—that is, at low concentrations of substrate the slope of a curve of reaction velocity plotted against substrate concentration increases as substrate concentration increases. Ordinary first-order Michaelis kinetics leads to a hyperbolic curve, in which the slope decreases steadily with increase in substrate concentration; a sigmoid response requires that more than one molecule of substrate interact in a rate-determining way with each molecule of enzyme. The addition of effector typically does not change the maximum reaction rate significantly, but shifts the whole pattern markedly either to the left (positive effector) or to the right (negative effector). It will be seen that for such enzymes the slope of the curve for rate versus substrate concentration at intermediate velocities remains steep, although it moves along the substrate-concentration axis. It seems reasonable to guess that this virtual constancy of slope is important in metabolic regulation and that this kinetic pattern is maintained in each case by selective pressure.

Almost nothing is known concerning the actual molecular basis for modulation of an enzyme's kinetic behavior by interaction with a small molecule. This important question, which has been the subject of much speculation (*4*), is outside the scope of this article. I will only repeat the suggestion (*1*, *5*) that modification caused by effectors [discovered by Umbarger (*2*) and Yates and Pardee (*3*)] and conformational changes caused by binding of substrate [deduced from kinetic data by Koshland (*6*)] should be considered as representatives of a single family of sterically specific and functionally significant interactions between proteins and small molecules. The widely used adjective "allosteric" was introduced by Monod and Jacob (*7*) to emphasize the important fact that modulation by an effector is different from competitive inhibition by an analogue of the substrate. The word has since been employed in different contexts with a variety of meanings, and at present is often used to denote nearly any interaction between a small molecule and a protein that leads to results other than (or in addition to) catalysis of a reaction in which the small molecule participates directly. In this sense most of the systems discussed in this paper can be termed allosteric.

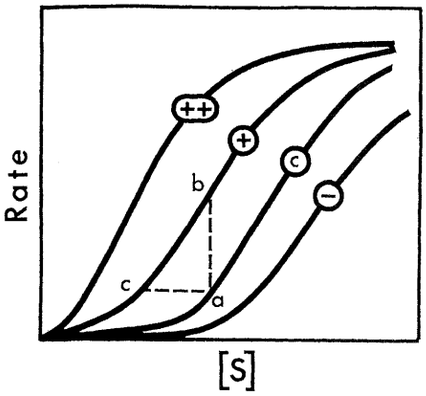

Fig. 1. Generalized substrate response curves for a regulatory enzyme. The rate of the reaction catalyzed by the enzyme is plotted on the vertical coordinate, and substrate concentration on the horizontal. Curve identifications: *c*, control; —, negative effector added; +, positive effector added; + +, higher concentration of positive effector. For significance of lettered points, see text.

Since substrate concentration is ordinarily considered an independent variable, the addition of a positive effector at some point *a* on the control curve of Fig. 1 would be expected to accelerate the reaction to the rate corresponding to point *b* on the curve for the concentration of effector present. However, if the velocity were held constant by a limitation on the rate at which substrate was supplied to the system, the addition of effector at point *a* would change the properties of the system to those indicated by point *c*. The rate of the reaction would still be determined by the rate of substrate supply, but the steady-state substrate concentration would be much lower than before. In intermediate cases, the addition of effector might lead to a velocity-substrate relationship given by any point on the curve between *b* and *c*. More generally, if the metabolic coupling in an intact cell is such that an increase in reaction rate leads to a decrease in concentration of the effector, the new situation may correspond to any point in the area *abc*; the actual result will depend on the relationship between parameters of the overall metabolizing system.

Viewed in this way, such enzyme kinetic curves may be considered analogous to engineering performance curves for various types of nonlinear control elements; in particular, the modulation of enzyme kinetics by effector concentration resembles the modulation of plate current by the voltage applied to the control grid of a vacuum tube (*8*).

Adenylates as Regulatory Effectors

Most metabolic energy-converting or energy-coupling processes occur at the expense of adenosine triphosphate (ATP), which is converted to adenosine-5′-monophosphate (AMP) or adenosine diphosphate (ADP). Regeneration of ATP is one of the major functions of both oxidative and fermentative metabolism. Since the concentrations of AMP and ATP will be inversely related in the intact cell, stimulation by AMP corresponds in metabolic terms to negative feedback by ATP. A number of recent observations suggest that both inhibition by ATP and stimulation by AMP play important roles in the regulation of energy metabolism. His earlier observations on phosphofructokinase of a parasitic liver fluke (*9*) led Mansour (*10*) to the discovery that both ordinary adenylic acid (adenosine-5′-monophosphate, AMP) and a cyclized isomer (adenosine-3′,5′-monophosphate) act as positive effectors for phosphofructokinase from guinea pig heart. Similar effects of AMP have been reported for phosphofructokinases from other tissues and organisms (*11*, *12*).

Hathaway and Atkinson (*13*), following up a report (*14*) that AMP is required for activity of yeast isocitrate dehydrogenase specific for diphosphopyridine nucleotide (DPN), found that AMP acts as a positive effector. In the presence of AMP the affinity of the enzyme for its substrate, isocitrate, is increased in the manner illustrated by Fig. 1. Indeed, an increase in the concentration of any component of the reaction (isocitrate, DPN^+, Mg^{++}, or AMP) was found to increase the apparent affinity of the enzyme for each of the others. Bernofsky and Utter have confirmed these results with the dehydrogenase activity retained in carefully isolated yeast mitochondria (*15*), and the same enzyme from other sources has generally similar properties (*16*), except that in animal cells so far examined ADP replaces AMP as the effector.

The kinetic properties of citrate syn-

thase, which catalyzes the formation of citrate from acetyl coenzyme A (acetyl-CoA) and oxaloacetate, have recently been shown to be regulated by ATP (*17*). An increase in the concentration of ATP strongly decreases the affinity of the enzyme for acetyl-CoA.

Phosphofructokinase, isocitrate dehydrogenase, and citrate synthase all participate in the expenditure of carbohydrate that is associated with the regeneration of ATP by oxidative phosphorylation; thus regulation of these enzymes by AMP and ATP should help to hold the rate of substrate utilization in step with the metabolic demand for ATP. Figure 2 illustrates schematically the suggestion (*12, 13*) that the course of energy metabolism may be controlled predominantly by the intracellular concentrations of AMP, ADP, and ATP. In the interest of clarity, intermediates not necessary to the discussion are omitted. The closed loop near the lower left-hand corner represents the Krebs citric-acid cycle.

The interrelationships diagrammed in Fig. 2 may be illustrated by considering first the limiting or boundary case of a cell which is essentially energy-saturated. This situation might arise, for example, when carbohydrates or other energy-yielding substrates were abundant but nitrogen was absent. The resultant inability of the cell to synthesize protein and carry out other energy-requiring growth processes would lead to accumulation of ATP. This compound is a negative effector for citrate synthase, which catalyzes the entry of acetyl-CoA into the Krebs cycle; thus under these conditions the cycle will compete weakly for acetyl-CoA.

When the concentration of ATP is high, the AMP concentration is necessarily low, and isocitrate dehydrogenase kinetics will resemble those represented by the control curve of Fig. 1. Even though the rate of the Krebs cycle reactions will be low under these conditions, the concentration of isocitrate should be relatively high. The concentration of citrate must exceed that of isocitrate by at least the equilibrium ratio of about 15. Citrate increases the substrate affinity of acetyl-CoA carboxylase, the enzyme catalyzing the first step in the conversion of acetyl-CoA to fat (*18*). It thus seems likely that this pair of regulatory enzymes working together may determine the relative tendency for acetyl-CoA to be used for fat production. The expected result is that a low concentration of AMP will cause fatty acid biosynthesis to compete more strongly for acetyl-CoA at the same time that the Krebs cycle, because of the high concentration of ATP, competes more weakly.

These coordinated effects presumably constitute at least part of the mechanism by which the cell partitions acetyl groups between immediate oxidation for ATP regeneration (the Krebs cycle) and deferred utilization (storage as fat). Under the conditions just described, the balance is in favor of fat storage. If the rate of expenditure of ATP increases, the resultant lowering of the ATP/AMP ratio will decrease the tendency for acetyl-CoA to be converted to fat and simultaneously increase its tendency to enter the Krebs cycle. This change is in the appropriate direction, since the oxidative reactions of the Krebs cycle are involved in the regeneration of ATP.

It might appear from Fig. 2 that production of fat when the concentration of ATP is high represents only a conversion of carbon from one storage form (polysaccharide) to another (fat). However, the situation is far more complex. The pathways shown represent to a considerable degree the main stream of energy metabolism, and other compounds, such as the amino acids derived from dietary proteins, are generally metabolized to intermediates in the pathways shown. Thus glutamic and aspartic acids, two of the most abundant components of proteins, are converted to α-ketoglutarate and oxaloacetate, respectively. In a reaction not shown here, oxaloacetic acid is decarboxylated to form pyruvic acid, which is then converted to acetyl-CoA and, under appropriate conditions, to fat. Some other amino acids are metabolized more directly to pyruvate or to acetyl-CoA, and thus can be utilized either for regeneration of ATP or for production of storage compounds.

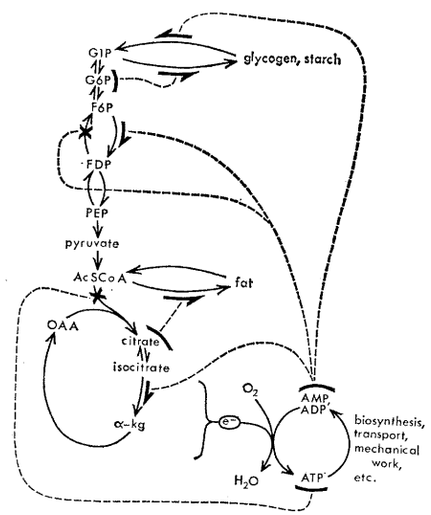

Fig. 2. Schematic illustration of the role proposed for AMP, ADP, and ATP in regulation of energy metabolism. Broken lines connect effector compounds (indicated by heavy arcs) to the enzymes which they modulate. Positive effector action is denoted by a heavy arrow, and negative effector action by a heavy cross. Abbreviations in addition to those defined in the text are: G1P, glucose-1-phosphate; G6P, glucose-6-phosphate; F6P, fructose-6-phosphate; FDP, fructose-1, 6-diphosphate; PEP, phosphoenolpyruvate; AcSCoA, acetyl coenzyme A; α-kg, α-ketoglutarate; OAA, oxaloacetate. Supply of electrons (from oxidative reactions in the glycolytic and Krebs cycle pathways) to the electron transport phosphorylation system is indicated by the symbol e^-.

As was noted above, AMP modulates the catalysis by phosphofructokinase of the conversion of fructose-6-phosphate to fructose diphosphate. The kinetic pattern is very similar to that of isocitrate dehydrogenase, except that the positive effector action of AMP on phosphofructokinase is augmented by a negative effector action of ATP (not shown in Fig. 2). By an analysis very similar to that outlined for isocitrate dehydrogenase, we may predict that in a cell with high ATP and low AMP concentrations the kinetic behavior of phosphofructokinase will be such as to lead to high concentrations of fructose-6-phosphate and its precursor, glucose-6-phosphate.

Glucose-6-phosphate, in turn, has been shown (*19*) to facilitate the action of the enzyme that catalyzes transfer of glucosyl groups to growing polysaccharide chains. Thus in these energy-rich cells the storage of polysaccharide, as well as of fat, will be facilitated. This result is enhanced by the action of ATP and AMP on fructose diphosphate phosphatase, which catalyzes hydrolysis of fructose diphosphate with the production of fructose-6-phosphate. This reaction is metabolically the reverse of the phosphofructokinase reaction and, as seems appropriate, it is inhibited by AMP and stimulated by ATP (*20*). Thus the conversion of phosphoenolpyruvate to polysaccharide will be favored in energy-rich cells. A rise in AMP concentration, signaling the need for ATP generation, will (i) inhibit fructose diphosphate phosphatase, thereby impeding the flow of later intermediates back toward polysaccharide; and (ii) change

the kinetic behavior of phosphofructokinase in such a way as to decrease the concentrations of fructose-6-phosphate and glucose-6-phosphate, which will render conditions for the conversion of hexose phosphate to polysaccharide less favorable. At the same time, AMP increases the activity of polysaccharide phosphorylase, which catalyzes the production of glucose-1-phosphate from storage polysaccharides. This regulatory effect of AMP was discovered by Cori, Colowick, and Cori in 1938 (*21*).

It was thus probably the first such effect to be observed, although a specific metabolic regulatory role was not proposed at the time. The phosphorylase system has since been intensively studied, notably in Cori's laboratory and by Fischer and Krebs and their colleagues (*22*), and it is at present the most complex regulatory system known. Its discussion is beyond the scope of this article; for our purposes it is sufficient to note the action of AMP as a positive effector.

In addition to their similar responses to AMP, the isocitrate dehydrogenase and phosphofructokinase regulatory systems are more directly interconnected, at least in some types of cells, by the action of citrate as a negative effector for phosphofructokinase (*23*).

Figure 2 may be considered as a first step toward the production of a regulatory metabolic map corresponding to the maps that have been prepared to show metabolic sequences. Like them, it is summary and superficial rather than specific and detailed. It does not take intracellular compartmentation into account, and the effects shown have not all been demonstrated in a single type of cell. It shows how several observed enzyme modulations may function in determining the momentary balance between oxidative metabolism and energy storage, and illustrates the hypothesis that the concentration of AMP is a major metabolic control variable. If the interrelationships suggested by Fig. 2 remain tenable, it is reasonable to expect that AMP will be found to modulate the action of other enzymes such as those that catalyze first steps in the degradation of optional energy sources. A high concentration of AMP might thus serve as an emergency signal causing amino acids, for example, to be diverted from their normal role in protein synthesis when the energy needs of the cell are extreme. The positive effector action of AMP and ADP on threonine and serine dehydrases (*24*) may be an example of this type of regulation.

A much more inclusive map could already be drawn on the basis of present knowledge. Many cell constituents, including purines, pyrimidines, and most amino acids, are biosynthesized from intermediates of this pathway, and in nearly all cases an early step in the synthesis has been shown to be governed by feedback control, usually with the end product serving as the negative effector. At least suggestive evidence is also available regarding the regulation of other metabolic processes by similar mechanisms.

Enzyme Repression

Feedback control of the kinetic behavior of enzymes (of the type discussed in this paper) is augmented by a different type of regulation known as enzyme repression—control of the rate of synthesis of an enzyme and thus of its concentration in the cell. Typically, the synthesis of an enzyme slows or stops altogether in response to an increase in the concentration of a specific metabolite—for example, the product of a synthetic sequence in which the enzyme participates. Feedback control usually involves only one enzyme in a metabolic pathway; in contrast, many or all of the enzymes of a sequence are often repressed simultaneously. Such a situation is termed "coordinate repression." Enzyme repression and the biochemical and genetic hypotheses proposed to explain it have been extensively reviewed (*1, 25*). The subject is introduced here only as a basis for discussion of possible interactions between feedback regulation and repression.

Both feedback inhibition of an enzyme active early in the pathway and coordinate repression of this same enzyme and later ones frequently occur in the same pathway. Such proliferation of control systems has generally been taken to represent solutions to two separate, though related, problems: (i) fine regulation of metabolic rates through the feedback effect and (ii) economy in enzyme synthesis as a consequence of repression. A very interesting paper by McFadden and Howes (*26*) proposes a closer relationship between these phenomena. Their paper dealt with the case of two enzymes catalyzing a step common to two sequences and both under feedback regulation, one by one of the two products and the second by the other. In this case coordinate repression of one of the enzymes catalyzing the common step and of the enzymes in one of the branch pathways could be advantageous in minimizing interdependence between the controls exerted by the products.

It appears that a type of interrelation between feedback and repression controls similar to that proposed by McFadden and Howes may be of interest in a more general context, without regard to branching of sequences. We retain their assumption that in the intact cell the rates of enzymic reactions depend on the activities of both enzyme and substrate (that is, that the enzymes are not kinetically saturated). In a system with feedback control of the first step and coordinate repression of the enzymes responsible for this and later steps, the following time sequence of control may be suggested: an increase in the concentration of product (for example, through exogenous supply) would lead to feedback inhibition of the first enzyme with a consequent decrease in steady state concentrations of all intermediates (since a lower rate of each reaction is now being catalyzed by the same amount of enzyme). On a longer time scale, however, all the enzymes in the pathway would be coordinately repressed. This should lead to an increase in the concentrations of all intermediates back toward the original steady-state levels (in comparison with the original situation, a smaller amount of each enzyme is now catalyzing a lower rate of reaction). These effects are represented schematically as case A in Fig. 3. If an intermediate, rather than the end product, should become available from the outside, the situation is quite similar (Fig. 3, case B). The concentration of the exogenously available intermediate will initially rise, since it is being supplied both from the outside and by continued biosynthesis. The concentrations of later intermediates and of the end product will of course also tend to increase. Feedback inhibition exerted by the product on the first step should, however, decrease the rate of this reaction until the rate of production of product, from both external and internal sources, approaches the original level. Coordinate repression of all enzymes in the pathway, with consequent partial relaxation of feedback inhibition at the first step, will lead to an even closer approximation of the orignal concentration profile. The analogy

between this case and an electronic feedback system is particularly obvious, since diffusion of an intermediate into the cell may be considered equivalent to an error signal, the consequences of which are minimized by operation of the feedback system.

A steady-state system may also be perturbed by increased demand for the product, leading to a decrease in its concentration, and by diminishing the extent of feedback control, leading to increased activity of the first enzyme. The concentrations of early intermediates will tend to increase (Fig. 3, case C). However, coordinate derepression (increase in the activity of all of the enzymes) would be expected to restore metabolite concentrations to essentially the original levels. Two of the various possibilities are illustrated for case C. The lower series of profiles is for a situation in which the steady-state concentrations of the intermediates are near the equilibrium ratios for the reactions in which they participate and all enzymes are present at kinetically equivalent levels. The upper profiles represent the situation where at least one step in the pathway (here taken as the last) is essentially unidirectional. In this case, which appears more likely, all intermediates prior to the physiologically irreversible step will tend to rise or fall together.

A simple unbranched biosynthetic sequence was used in Fig. 3 to illustrate the hypothesis that concentrations of metabolites are maintained at nearly steady levels by joint action of repression and feedback controls. The hypothesis, however, is general; if it is valid, it probably applies to the whole complex network of metabolic reactions. It seems possible that this sort of interrelation between regulatory mechanisms might be involved in a wide variety of physiological adaptations, including changing response to therapeutic agents and even perhaps addiction to narcotics. If the primary effect of a drug is to alter the rates of several enzyme-catalyzed reactions, a serious imbalance in concentrations of metabolites may result. Repression and related mechanisms would be expected to lead to changes in the activities of enzymes participating in many relevant sequences in such a way as to minimize the metabolic disruptions caused by the drug and to favor survival of the cell. Such changed activities, however, must necessarily be deleterious in the absence of the stimulus that evoked them. The more extreme the alteration of activities in a drug-adapted cell or organism, the more poorly adjusted it will become to cope with normalcy. In such cells the absence of the drug may lead to metabolic imbalances as drastic as those initially caused by massive doses.

Control Sensitivity

If feedback and repression controls interact in the way just proposed, repression will need to be a very sensitive response, since it must be activated by the small residual change remaining after the primary fluctuation in concentration has been minimized by feedback control. Equally obviously, feedback control must be sensitive (the whole useful range of response must occur over a narrow range of effector concentrations) if regulatory interactions are to be effective. This expectation is confirmed by studies in vitro. Most regulatory enzymes give sigmoid curves not only when rate is plotted against substrate concentration, but also when rate is plotted against effector concentration. As Changeux (*27*) pointed out, there is thus a threshold concenration below which the effector has little effect. Above this threshold, a wide range of response is typically evoked by small variation in effector concentration. It is obvious that the operational range of each effector is a fundamental regulatory parameter that must be under as strict evolutionary constraint as is any other property of the enzyme molecule.

High sensitivity of control systems is probably advantageous to the cell, but it adds to the difficulties faced by the metabolic chemist. Hypotheses such as that outlined in Fig. 2 need to be tested in the living cell, and an obvious approach is to find whether concentrations of the metabolites involved vary in the expected ways in response to changes, for example, in the availability of oxygen, energy-yielding substrates, or metabolizable nitrogen compounds. But the more effective the regulation, the smaller the changes in concentration; to make matters worse,

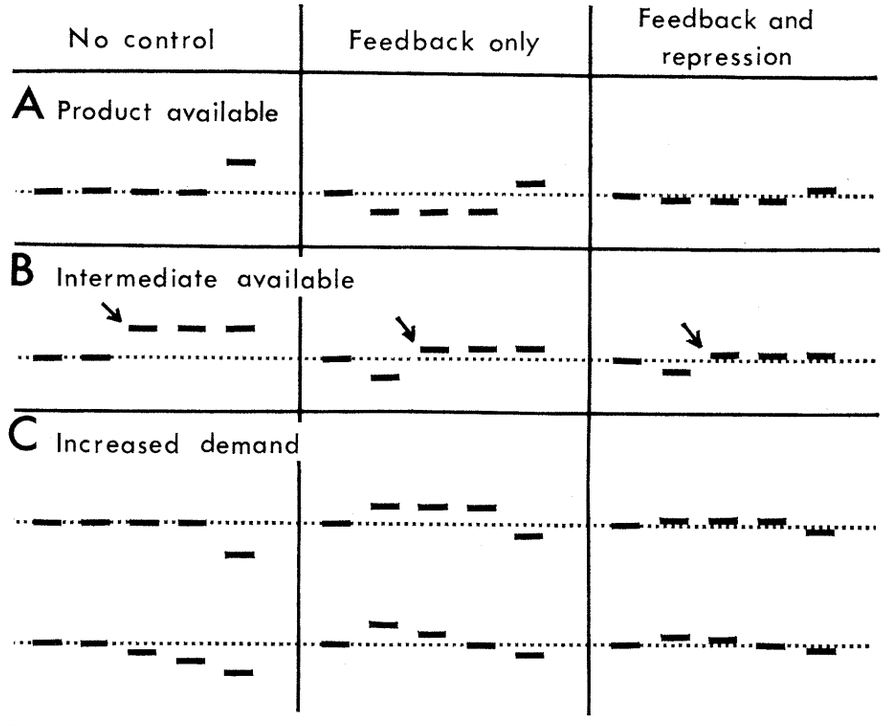

Fig. 3. Suggested interaction between feedback control and enzyme repression (schematic representation). The heavy line segments indicate the concentrations of five successive intermediates in a biosynthetic sequence under the conditions indicated. The steady-state concentration of each intermediate before the system was perturbed (by external availability of product or an intermediate, or by increased metabolic demand for the product) is represented arbitrarily by the broken line. There is, of course, no implication that the concentrations of the various intermediates would be equal. Concentrations relative to these prior steady-state levels are indicated by the vertical positions of the line segments. (A vertical concentration scale is assumed.) The figure illustrates the hypothesis that the joint action of feedback and repression controls tends to restore the original concentration profile.

concentrations are not likely to be uniform throughout a cell, and the average level of a substance in the cell may remain nearly constant even if the concentration in the vicinity of a regulatory enzyme changes appreciably. One naturally tends to assume that those metabolites whose intracellular concentrations are observed to change most markedly are by that token the most likely to be involved in metabolic regulation, but the opposite view could be argued as persuasively. A man who had never seen a thermostatic water bath might reason from an analysis of its construction that the observable fluctuations in current through the heaters were controlled by minute changes in water temperature, but unless he had a sensitive thermometer he could not confirm his hypothesis by direct experiment. We cannot measure the concentrations of effectors in the vicinity of enzymes in the living cell with precision corresponding to that of a good thermometer. Even when the concentration of a regulating metabolite does vary widely in the cell, its effective regulatory range may be much narrower, just as the potential applied to a control grid may swing far below the tube's cut-off voltage.

Generalizations

A number of observations and generalizations related to the topics discussed will be touched on briefly.

Feedback regulation of individual enzymes may be much more complex than in the cases discussed here. Datta and Gest (*28*) have reported that aspartokinase of *Rhodopseudomonas capsulatus* is affected very little by either threonine or lysine (both of which are synthesized by way of the aspartokinase reaction) but is very strongly inhibited when both are present simultaneously. Gest named this type of response "concerted feedback inhibition." A contrasting type of multiple response was observed by Woolfolk and Stadtman (*29*) in the case of glutamine synthetase of *Escherichia coli*. This enzyme participates in the biosynthesis of many metabolites, and eight of them were found to inhibit its activity. Each effector inhibits only to a small extent, and they seem to act independently; that is, when two or more are present simultaneously, the fraction of normal activity observed is approximately the product of the fractions obtained with each individually. The term "cumulative feedback inhibition" was proposed for such interactions. It is to be expected that more examples of these complex patterns, as well as new types, will be discovered.

Feedback effects augment mass action. In every case of which I am aware, metabolic feedback regulation acts in the same direction as would simple mass action control. In some cases mass action effects may only be amplified, but more typically feedback action transmits the effect past a physiologically irreversible step (where mass action effects would be negligible) by direct modulation of the enzyme catalyzing the irreversible reaction. Since feedback control seems much more powerful than mass action, it might be considered of little consequence whether the two effects act in concert or in opposition. Reinforcement of mass action was probably critical for the evolution of feedback control, however. Furthermore, feedback controls all seem directed toward maintenance of relatively constant concentrations of metabolites; thus the end result and the metabolic advantages of these controls may be seen in terms of mass action considerations.

Metabolic sequences are largely unidirectional. This generalization did not arise from work on regulation; its importance has gradually come to be appreciated during the past several years. But unidirectionality and feedback control are closely related.

In nearly every known case where a metabolite A can be converted to B and B can also be converted to A, the conversions proceed by different pathways with different stoichiometries and therefore different equilibrium relationships. Each sequence is usually unidirectional because it contains at least one reaction that is physiologically irreversible—that is, a reaction with an equilibrium constant sufficiently large that the reverse reaction will never occur in a living cell. The difference in stoichiometries usually involves ATP, which, as mentioned above, is the primary energy mediator in metabolism. Thus in examples from Fig. 2, a molecule of ATP is expended for each molecule of glucose-1-phosphate incorporated (as a glucosyl residue) into glycogen or starch, but no ATP is gained when glucose-1-phosphate is regenerated from the polysaccharide. Both reactions, therefore, are simultaneously thermodynamically favorable. Many more molecules of ATP are used in the production of each molecule of fatty acid from acetyl-CoA than are produced when the fatty acid is degraded to acetyl-CoA. In both of these cases, the intermediates and enzymes of the oppositely directed sequences are entirely different. In the third such case shown in Fig. 2—the conversion of hexose phosphate to phosphoenolpyruvate and vice versa—it is generally assumed that most of the enzymes function in both directions, although this is not certain. But one interconversion—that between fructose-6-phosphate and fructose-1,6-diphosphate—is known to be catalyzed by two different enzymes that are in physiological terms oppositely directed. The two reactions are (ignoring ionizations):

$$\text{fructose-6-phosphate} + \text{ATP} \rightarrow \text{fructose-1,6-diphosphate} + \text{ADP}$$

$$\text{fructose-1,6-diphosphate} + \text{H}_2\text{O} \rightarrow \text{fructose-6-phosphate} + \text{H}_3\text{PO}_4$$

The equilibrium constant for each of these reactions is large in the direction written, and it is inconceivable that the products of either could accumulate in a living cell to a point allowing reversal. Enzymes for both are present in most cells. Thus if there were no control on the activities of these enzymes, both reactions would occur in the thermodynamically favored direction as shown, and the net reaction would be merely

$$\text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{H}_3\text{PO}_4$$

which would amount to a short-circuit in the cell's energy metabolism. This fatal result is prevented by the control of both enzymes by AMP and ATP as discussed in connection with Fig. 2 (and possibly by additional mechanisms yet to be discovered). The advantages of what we may call "irreversibility loops"—oppositely oriented unidirectional sequences—appear obvious. The cell can mobilize its stored reserves quickly when the need arises instead of having to deplete its metabolic pools until storage comes to a stop for equilibrium reasons and the polysaccharide or fat begins sluggishly to break down by reversal of the storage pathway. If only because the concentrations of metabolic intermediates would be lowest under emergency conditions—when storage compounds were being utilized—an organism that functioned in this way would have little evolutionary future. It may well be that the most important single consequence of feedback control is that it makes irreversibility loops possible.

Present Status

Our present situation with regard to understanding metabolic control might be compared to that of a visitor from a different culture who, though not quite able to comprehend the regulation of a thermostatic water bath, tries without guide or guidebook to understand the control system of a modern satellite. We can rationalize in terms of apparent regulatory function the observation that the catalytic activities of several enzymes are modulated by metabolites. The metabolic relationships between the regulatory metabolites and the enzymes, and the interrelations between the enzymes that are so modulated, are such as to give us a high degree of confidence that our rationalizations are at least partially correct. But most of the apparently regulatory enzymes, as studied in vitro, are strongly influenced also by changes in *p*H, and many of them by the concentrations of various cations. Since we have no way of estimating the extent to which the local *p*H or activities of cations may vary in the cell, we cannot even guess to what extent these parameters participate in metabolic regulation. It seems likely that in the intact cell most enzymes are attached to structural elements and so have a more or less definite spatial relationship to other enzymes. The observed kinetic modulations of regulatory enzymes are generally assumed to be accompanied by changes in the conformations of the enzyme molecules. Such changes must alter the relation of an enzyme to other enzymes as well as to other functional elements in the cell, and alterations within a living cell are not likely to occur randomly. It would be surprising if regulatory enzymes had not evolved in such a way that the physical consequences of effector modulation would reinforce the chemical aspects of regulation. Life is notable for the intensity and complexity of its component chemical reactions, but a more specific characteristic is its harmonious self-regulation in the face of drastic changes in external conditions and of the still more drastic threat of internal chaos. This regulation must require far more in the way of molecular and supramolecular specificity than does the simple catalysis of the reactions themselves; and it is likely that most of the genetic information transmitted to daughter cells relates to this area of which we as yet understand almost nothing. Metabolic chemistry is in its infancy. To paraphrase Newton's famous metaphor, we have amused ourselves with the shiny pebbles of metabolic sequences (and the smaller pebbles of individual enzymic reactions), while before us lay largely unperceived the ocean of interrelation and regulation. We do not yet really understand any of our pebbles, and we have only begun to notice a bit of salt spray in the air.

References and Notes

1. H. E. Umbarger, *Science* **145**, 674 (1964), and papers cited there.
2. ———, *ibid.* **123**, 848 (1956); see also ——— and B. Brown, *J. Biol. Chem.* **233**, 415 (1958).
3. R. A. Yates and A. B. Pardee, *J. Biol. Chem.* **221**, 757 (1956).
4. J. C. Gerhart and A. B. Pardee, *ibid.* **237**, 891 (1962); J. Monod, J. P. Changeux, F. Jacob, *J. Mol. Biol.* **6**, 306 (1963); C. Frieden, *J. Biol. Chem.* **239**, 3522 (1964); J. C. Gerhart and A. B. Pardee, *Federation Proc.* **23**, 727 (1964); D. E. Atkinson, J. A. Hathaway, E. C. Smith, *J. Biol. Chem.* **240**, 2682 (1965); M. Klingenberg, H. Goebell, G. Wenske, *Biochem. Z.* **341**, 199 (1965); J. Monod, J. Wyman, J. P. Changeux, *J. Mol. Biol.* **12**, 88 (1965).
5. D. E. Atkinson and G. M. Walton, *J. Biol. Chem.* **240**, 757 (1965).
6. D. E. Koshland, in *Horizons in Biochemistry*, M. Kasha and B. Pullman, Eds. (Academic Press, New York, 1962), p. 265; *Cold Spring Harbor Symp. Quant. Biol.* **28**, 473 (1963); *Science* **142**, 1533 (1963); *Federation Proc.* **23**, 719 (1964).
7. J. Monod and F. Jacob, *Cold Spring Harbor Symp. Quant. Biol.* **26**, 389 (1961).
8. It is important to note that the response to effector concentration is continuously variable; that is, an infinite family of curves of the type shown in Fig. 1 could be obtained by using intermediate levels of effector. In this important respect the regulated enzyme resembles such technological control devices as vacuum tubes or transistors rather than on-off devices such as switches or relays. The individual enzyme molecule may have a finite number of stable conformations (possibly a function of the number of substrate and effector binding sites). But because the rates at which substrate and effector molecules are adsorbed and desorbed are very high compared to the time scale of observation or of metabolic regulation (and perhaps even compared to the relaxation times associated with interconversions among enzyme conformations), the effective kinetic properties of even a single enzyme molecule may be expected to vary continuously with effector concentration. It is still more obvious that the composite properties of the many molecules of a given enzyme in an assay mixture or a living cell must be capable of continuous variation.
9. T. E. Mansour and J. M. Mansour, *J. Biol. Chem.* **237**, 629 (1962).
10. T. E. Mansour, *ibid.* **238**, 2285 (1963).
11. J. V. Passonneau and O. H. Lowry, *Biochem. Biophys. Res. Commun.* **7**, 10 (1962).
12. A. Ramaiah, J. A. Hathaway, D. E. Atkinson, *J. Biol. Chem.* **239**, 3619 (1964).
13. J. A. Hathaway and D. E. Atkinson, *ibid.* **238**, 2875 (1963).
14. A. Kornberg and W. E. Pricer, Jr., *ibid.* **189**, 123 (1951).
15. C. Bernofsky and M. F. Utter, Abstracts, Sixth International Congress of Biochemistry, 1964, p. 297.
16. R. F. Chen and G. W. E. Plaut, *Biochemistry* **2**, 1023 (1963); B. D. Sanwal, M. W. Zink, C. S. Stachow, *J. Biol. Chem.* **239**, 1597 (1964); B. D. Sanwal and C. S. Stachow, *Biochim. Biophys. Acta* **96**, 28 (1965); H. Goebell and M. Klingenberg, *Biochem. Z.* **340**, 441 (1964).
17. J. A. Hathaway and D. E. Atkinson, *Biochem. Biophys. Res. Commun.* **20**, 661 (1965).
18. D. B. Martin and P. R. Vagelos, *J. Biol. Chem.* **237**, 1787 (1962); M. Waite and S. J. Wakil, *ibid.*, p. 2750; A. F. Spencer and J. M. Lowenstein, *ibid.*, p. 3640; P. R. Vagelos, A. W. Alberts, D. B. Martin, *ibid.* **238**, 533 (1963).
19. L. F. Leloir, J. M. Olavarria, S. H. Goldemberg, H. Carminatti, *Arch. Biochem. Biophys.* **81**, 508 (1959); I. D. Algranati and E. Cabib, *Biochim. Biophys. Acta* **43**, 141 (1960).
20. K. Taketa and B. M. Pogell, *Biochem. Biophys. Res. Commun.* **12**, 229 (1963); ———, *J. Biol. Chem.* **240**, 651 (1965); E. A. Newsholme, *Biochem. J.* **89**, 38p (1963); H. A. Krebs, E. A. Newsholme, R. Speake, T. Gascoyne, P. Lund, in *Advances in Enzyme Regulation*, G. Weber, Ed. (Macmillan, New York, 1964), vol. 2, p. 71; M. Salas, E. Viñuela, J. Salas, A. Sols, *Biochem. Biophys. Res. Commun.* **17**, 150 (1964); W. L. Byrne, E. Hall, T. M. Harris, A. M. Geller, L. R. Reid, *Federation Proc.* **24** (abstract), 356 (1965).
21. G. T. Cori, S. P. Colowick, C. F. Cori, *J. Biol. Chem.* **123**, 381 (1938).
22. D. H. Brown and C. F. Cori, in *The Enzymes*, P. D. Boyer, H. Lardy, K. Myrbäck, Eds. (Academic Press, New York, 1961), vol. 5, p. 207; E. G. Krebs and E. H. Fischer, *Advance. Enzymol.* **24**, 263 (1962).
23. P. B. Garland, P. J. Randle, E. A. Newsholme, *Nature* **200**, 169 (1963); A. Parmeggiani and R. H. Bowman, *Biochem. Biophys. Res. Commun.* **12**, 268 (1963); J. V. Passonneau and O. H. Lowry, *ibid.* **13**, 372 (1963).
24. W. A. Wood and I. C. Gunsalus, *J. Biol. Chem.* **181**, 171 (1949); H. E. Umbarger and B. Brown, *J. Bacteriol.* **73**, 105 (1957); M. Tokushige, H. R. Whiteley, O. Hayaishi, *Biochem. Biophys. Res. Commun.* **13**, 380 (1963); A. T. Phillips and W. A. Wood, *Biochem. Biophys. Res. Commun.* **15**, 530 (1964); M. Hirata, M. Tokushige, A. Inagaki, O. Hayaishi, *J. Biol. Chem.* **240**, 1711 (1965).
25. H. J. Vogel, in *The Chemical Basis of Heredity*, W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, 1957), p. 276; F. Jacob and J. Monod, *Cold Spring Harbor Symp. Quant. Biol.* **26**, 193 (1961); ———, *J. Mol. Biol.* **3**, 318 (1961); G. S. Stent, *Science* **144**, 816 (1964); B. N. Ames and R. G. Martin, *Ann. Rev. Biochem.* **33**, 235 (1964); W. K. Maas and E. McFall, *Ann. Rev. Microbiol.* **18**, 95 (1964).
26. B. A. McFadden and W. V. Howes, *Arch. Biochem. Biophys.* **109**, 415 (1965).
27. J. P. Changeux, *Cold Spring Harbor Symp. Quant. Biol.* **26**, 313 (1961).
28. P. Datta and H. Gest, *Proc. Nat. Acad. Sci. U.S.* **52**, 1004 (1964).
29. C. A. Woolfolk and E. R. Stadtman, *Biochem. Biophys. Res. Commun.* **17**, 313 (1964).

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