Airport. See: from class 202, dockets 8, 187, 235–257; from class 203, dockets 10, 11, 89–91; and other classes.
8. A. Normandy, U.S. Patent 21,693 (5 October 1858).
9. G. W. Baird, U.S. Patent 233,184 (12 October 1880).
10. T. Craney, U.S. Patent 524,887 (12 August 1894).
11. J. F. Witteman, U.S. Patent 845,286 (26 April 1907).
12. R. P. Barnstead, U.S. Patent 823,488 (12 June 1906).
13. E. E. Buscher, U.S. Patent 1,276,025 (20 August 1918).
14. R. E. Skow, U.S. Patent 3,055,810 (25 September 1962).
15. R. W. Powers, *Electrochem. Technol.* **2**, 163 (1964).
16. Traditional treatment, origin unknown; see the preamble of Powers (*15*).
17. O. Soderlund and T. Boberg, U.S. Patent 1,200,996 (10 October 1916); R. V. Kleinschmidt, U.S. Patent 2,185,595 (2 January 1940).
18. For information on the earliest U.S. plant, see "Saline Water Conversion Report" (U.S. Department of the Interior, Washington, D.C., 1961), pp. 88–91.
19. See, for instance, *Principles of Desalination*, K. S. Spiegler, Ed. (Academic Press, New York, 1966), pp. xiv and 566.
20. See history by C. H. Giles and S. D. Forrester, *Chem. Ind. London* **2**, 43 (1971).
21. K. Hickman, *Separ. Sci.* **3**, 551 (1968).
22. ———, U.S. Patent Application, Serial No. 244,155 (24 May 1972) assigned to U.S. Department of the Interior pursuant to contract 14-30-2572, "System for Producing and Maintaining Purified, Sterile Water" (section 1).
23. ———, U.S. Patent Application, Serial No. 281,667 (29 August 1972) assigned to U.S. Department of the Interior pursuant to contract 14-30-2572, "System for Producing and Maintaining Purified, Sterile Water" (section 2).
24. P. J. Harris, *J. Phys. Chem.* **74**, 2317 (1970).
25. J. C. Berg and A. Acrivos, *Chem. Eng. Sci.* **20**, 737 (1965) (see figures 4 and 5).
26. See, for instance, P. J. Wangersky, *Amer. Sci.* **53**, 358 (1965).
27. A swab taken from the inside of a window pane is likely to assay for the whole, ubiquitous gamut.
28. F. Kohlrausch and A. Heydweiller [see (*6*)]; A. Iverson, *J. Phys. Chem.* **68**, 515 (1964); H. C. Duecker and W. Haller, *ibid.* **66**, 225 (1962); M. Eigen and L. DeMaeyer, *Z. Elektrochem.* **55**, 989 (1955); C. G. Malmberg, *J. Res. Nat. Bur. Stand. Sect. A* **69**, 39 (1965); J. R. Fisher and H. A. Barnes, *J. Phys. Chem.* **76**, 90 (1972); K. Hickman *et al.* [see (*29*)].
29. K. Hickman, I. White, W. V. Kayser, H. Cammenga, A. J. Roggen, A. Davidhazy, G. A. Mosholder, "Further Observations on the Surface Properties of Water" [final report to the Office of Saline Water for grant 14-30-2572 (1972)].
30. Standard assays are commercially available from G. D. Brown, 24 Parkside Drive, Jamaica Plain, Massachusetts 02130.
31. We thank Dr. H. K. Cammenga, who gave us valuable information on the specific conductivity of water; Dr. W. Kayser, who refined the method of spiralizing plug flow and provided three photographs for Figs. 7 and 8; and A. Roggen, who assisted in the construction of the still and, with G. Mosholder, helped in the preparation of the manuscript. The work was supported by the Office of Saline Water under grants 14-30-2572 and 14-30-2964.

Enzymatic Interconversion of Active and Inactive Forms of Enzymes

A new mechanism of metabolic control has become evident.

Harold L. Segal

Every student of biochemistry has seen, and perhaps even looked at, two-dimensional metabolic maps that outline the myriad pathways of biodegradation and biosynthesis. He understands that for each arrow on the map pointing from one metabolite to another, one step modified, there exists a corresponding enzyme, which in principle can be named by inspection of the reaction. The virtual completion of the basic framework of such maps in all its detail has been a triumph of biochemistry, largely over the last quarter century, although occasionally a new addition not fully anticipated is still made.

Recently new kinds of arrows have begun to appear on such maps indicating that certain metabolites are also modulators of reactions in which they are not reactants or products. This new order of complexity, encompassed in the concept of modulator control, while certainly far from completely worked out, adds greatly to the reality that metabolic maps are intended to convey. The inclusion of this aspect of metabolic control requires the addition of essentially no new elements to the matrix. The same enzymes and the same metabolites and cofactors which comprise the pathways of metabolism themselves are the units which effect modulator control. It is only necessary to enlarge the concept of enzyme action to include in some cases modulator binding at sites distinct from the reaction sites. The map, however, is still two dimensional.

The experiments of the Coris and their co-workers 25 to 30 years ago, revealed that one of the enzymes present on metabolic maps, glycogen phosphorylase, exists in two forms, called *a* and *b* (*1*). With the discovery that these forms, which differ from one another covalently by the presence (*a* form) or absence (*b* form) of esterified phosphate groups, could be interconverted enzymatically, a new dimension of enzymology emerged. Phosphorylase *a* phosphatase, which catalyzes the *a* to *b* conversion, and phosphorylase *b* kinase, which catalyzes the *b* to *a* conversion, are of a different sort functionally from the enzymes that interconvert metabolites (metabolic enzymes). They are enzymes whose substrates are enzymes. The inference is that the interconversion of forms of metabolic enzymes has a control function, and there is substantial evidence to support this view.

In addition to the opportunities such systems provide for metabolic control, there are some genetic and developmental consequences as well in the possibilities for multigenic control of enzyme expression. Where an "activating enzyme" is required for the conversion of a metabolic enzyme, or other physiologically active protein, to its functional state, a failure of appearance of activity may not denote a lack of a functional structural gene for the protein in question, or even the absence of the protein itself. What may be lacking is the "activating enzyme." One wonders how often, in the catalogs that relate appearance of functional enzyme activity with embryological development, it is gene expression for an "activating enzyme" that is manifested rather than for the functional protein per se. Analogous questions can be

The author is a professor in the department of biology at the State University of New York, Buffalo 14214.

raised in regard to *loss* of functional activity, as, for example, with cells in culture or malignant cells. In at least one case of loss of functional enzyme activity with altered hormonal or nutritional state the defect is not a lack of the metabolic enzyme, but rather of one of the interconverting enzymes.

It is important to distinguish between the type of system being considered in this article and other types of systems with certain similarities. Isozymes, for example, are also sets of proteins with related catalytic properties and differences in covalent structure. Isozymes, however, differ from one another in internal amino acid sequences, reflecting separate structural genes, and are not interconvertible, whereas interconvertible forms arise from the same structural gene and differ by the presence or absence of groups other than internal amino acid residues. In addition, many enzymes exist in multiple states, which have distinct kinetic properties but which differ only conformationally, and are in thermodynamic equilibrium with one another [for some examples, see (*2*)]. There are cases in which proteins are covalently modified enzymatically but, unlike the interconvertible systems, only in one direction. Among these are the methylation reactions (*3*), the formation of active proteins from inactive precursors by selective proteolysis (*4*), the formation of collagen from protocollagen by hydroxylation and glycosylation (*5*), and the blood clotting process (*6*). Such reactions are usually viewed as elements of the overall pathway of functional protein synthesis rather than as aspects of metabolic control. They share with the bidirectional systems, however, the opportunity for multigenic control of enzyme expression. Table 1 lists a number of enzymes for which there is evidence, convincing or suggestive, for the existence of two forms, interconvertible by control enzymes.

The Phosphorylase System

As already mentioned, the phosphorylase system was the first of this type to be recognized and its analysis is furthest advanced, especially in muscle, notably from the work of Krebs and Fischer and their co-workers (*7*), although its complexity continues to unfold.

Phosphorylases can be divided roughly into two types, the skeletal muscle type, characterized by substantial activity of the *b* form relative to the *a* form in the presence of adequate concentrations of AMP (*8*), and the liver type, of which the *b* form has little activity even in the presence of AMP. The phosphorylases of other tissues fall into one or another of these general categories. Recent reviews of phosphorylase have appeared (*9*).

Muscle phosphorylase. Morgan and Parmeggiani and co-workers (*10, 11*) and Helmreich and Cori and co-workers (*12, 13*) have investigated the molecular basis of physiological control of heart and skeletal muscle phosphorylase. They have found that modulator effects of ligands and interconversion of *b* and *a* both play a role. In resting aerobic muscle, phosphorylase is almost entirely in the *b* form, which is virtually inactive in the presence of in vivo levels of control ligands in this state (P_i, AMP, ATP, ADP, G-6-P). Phosphorylase *a*, on the other hand, is highly active under these conditions so that *b* to *a* interconversion constitutes a switching of the system between on and off states. The stimulation of glycogenolysis by the hormones, glucagon and epinephrine, and by electrical stimulation can be attributed to this mechanism. Similarly in the reverse sense, the prevention by glucose or G-6-P of glycogen depletion in isolated diaphragm was correlated with a concomitant reduction in phosphorylase *a* (*14*). On the other hand, anoxia promotes an even more rapid flow through the phosphorylase reaction with a lesser level of phosphorylase *a* formation (*10*). In this case a substantial part of the effect is evidently due to a release of the restraint on phosphorylase *b* resulting from changes in the concentrations of P_i, AMP, ATP, ADP, and G-6-P (*11*).

Liver phosphorylase. As mentioned above, phosphorylase *b* of liver (also referred to as inactive phosphorylase and dephosphorylase) is inactive under physiological conditions, although activity can be elicited in vitro by the addition of high concentrations of sulfate. The *a* and *b* forms differ from one another by the presence or absence of esterified phosphate groups, respectively, and are interconvertible by kinase and phosphatase action as with the muscle enzyme. It was the studies with this system by Sutherland and Rall and their co-workers [see (*15*)] which led to the discovery of the existence and role of cyclic nucleotides in metabolic control.

The stimulation of glycogenolysis by the hormones glucagon and epinephrine is associated with a conversion of phosphorylase *b* to *a* through the mediation of cyclic AMP (*16*). Glucose produces the opposite effect on the phosphorylase system, in that it leads to a reduction

Table 1. Interconvertible enzymes and proteins.

Muscle phosphorylase	*b* activity repressed by endogenous modulators *b* form assayable in presence of AMP
Liver phosphorylase	*b* form inactive, activity not elicited by AMP
Phosphorylase *b* kinase	*b* form active in vivo at high Ca^{2+}, low ATP *b* form assayable at high *p*H
Phosphorylase *a* phosphatase	*a* and *b* forms appear to exist Activity stimulated by glucose
Muscle glycogen synthetase	*b* form activated in vivo by elevated G-6-P
Liver glycogen synthetase	*b* form inactive at tissue ligand concentrations, assayable in presence of G-6-P
Hormone-sensitive lipase	*b* to *a* reaction catalyzed in vitro by protein kinase Activated in vivo by lipolytic hormones
Pyruvate dehydrogenase	Phosphatase reaction (*b* to *a*) promoted by cyclic AMP Fatty acids promote *a* to *b* reaction in vivo
Escherichia coli glutamine synthetase	*a* to *b* conversion by adenylylation *b* form subject to feedback inhibition
P_{II} component of glutamine synthetase interconverting system	Separate forms catalyze glutamine synthetase activation and inactivation P_{II} conversion in only one direction demonstrated
Histones	Interconverted by both phosphorylation and acetylation Also phosphorylated by a cyclic GMP–stimulated kinase
RNA polymerase	In vivo significance of interconversion not established
Polynucleotide phosphorylase	In vivo significance of interconversion not established
Ribosomal proteins	In vivo significance of interconversion not established
Neurotubular proteins	In vivo significance of interconversion not established

in the amount of phosphorylase *a* (*17, 18*). In this case no change in cyclic AMP could be detected (*18*). Furthermore, the effect on the phosphorylase system of suboptimum doses of glucagon could be overcome by glucose without reducing the glucagon-induced increase in cyclic AMP (*17*).

Phosphorylase b *kinase.* The conversion of phosphorylase *b* to *a* involves a Mg^{2+}-dependent transfer of phosphate from ATP to one serine residue per subunit of phosphorylase *b*, catalyzed by phosphorylase kinase. Muscle phosphorylase kinase itself exists in two forms, one of which is active and the other inactive in the resting tissue (*19*). The latter can be assayed in vitro, however, if the determination is carried out at high *p*H (*20*). To maintain a consistency of nomenclature the active and "*p*H 7 inactive" forms will be referred to as phosphorylase kinase *a* and *b*, respectively. Phosphorylase kinase of liver also appears to exist in active and inactive forms (*21*).

Phosphorylase kinase of muscle has been purified to near homogeneity. Of a number of glycolytic enzymes of muscle tested as substrates, only phosphorylase *b* was phosphorylated (*22*). Recently, however, it has been reported that the inhibitor component of troponin, which is closely associated with actomyosin, can also be phosphorylated by phosphorylase kinase (*23*). In this case both forms of the kinase were equally effective.

The kinase can be converted to the *a* form in vitro in the presence of ATP and Mg^{2+}, and during this process there is a phosphorylation of the enzyme. To a minor extent the phosphorylation and activation appear to be self-catalyzed (*24*); however, both are greatly accelerated by small amounts of another kinase in muscle. It is this kinase in muscle which is the species directly affected by cyclic AMP (*25*). This enzyme, referred to as cyclic AMP–dependent protein kinase, is involved in a number of interconvertible enzyme systems as is discussed below.

The presence in extracts from a variety of tissues of an enzyme that dephosphorylates and inactivates phosphorylase kinase *a* has been demonstrated (*26*). The muscle enzyme was shown to be distinct from phosphorylase *a* phosphatase, as well as from phosphatases that catalyze the hydrolysis of casein phosphate and other phosphate esters of low molecular weight.

On the basis of these observations, plus the finding that administration of epinephrine leads to increases in phosphorylase *a*, phosphorylase kinase *a*, and cyclic AMP in muscle and heart (*27, 28*), the sequence of events shown in the upper part of Fig. 1 has been deduced to describe the catecholamine-induced promotion of glycogenolysis. One point to be noted here is that *two* interconvertible enzyme systems are involved in the pathway.

Table 2. Mechanisms of stimulation of the phosphorylase reaction.

Signal	Response
Epinephrine, glucagon	Cyclic AMP–mediated conversion of phosphorylase kinase *b* to *a*, leading to conversion of phosphorylase *b* to *a*
Electrical stimulation	Ca^{2+} stimulation of phosphorylase kinase *b*, leading to conversion of phosphorylase *b* to *a*
Anoxia	Change in modulator concentrations, leading to increased activity of phosphorylase *b*

On the other hand, direct electrical stimulation of muscle leads to a very rapid increase in phosphorylase *a* (*12*), with no increase in cyclic AMP or phosphorylase kinase *a* (*28*). The same pattern of response was obtained in perfused hearts by increasing the concentration of Ca^{2+} in the medium (*29*). In vitro evidence has been presented that Ca^{2+} stimulates phosphorylase kinase *b* directly (*30, 31*). In this way contraction and increased glycolysis can be directly linked via alterations in Ca^{2+} flux consequent to electrical stimulation, independent of cyclic AMP–dependent phosphorylase kinase *b* to *a* conversion (*28, 29, 31*). Thus there are three separate responses to three distinct signals all leading to an increased flow through the phosphorylase system (Table 2).

Phosphorylase a *phosphatase.* Conversion of phosphorylase *a* to *b* involves the hydrolysis of serine phosphate groups of the enzyme, catalyzed by a specific phosphatase. Active and inactive forms of the phosphatase, which I will refer to as phosphatase *a* and *b*, respectively, have been demonstrated in adrenal cortex, liver, and muscle (*32*). Conversion of the *b* to the *a* form requires ATP and Mg^{2+}.

Since the level of phosphorylase *a* in the tissue represents a balance between its formation via phosphorylase kinase and its removal via phosphorylase phosphatase, it is evident that the phosphorylase reaction can be modulated through either the kinase system, as has been discussed above, or the phosphatase system. The existence of active and inactive forms of phosphorylase phosphatase further suggests the possibility that the latter system is subject to physiological control. Direct evidence for the phosphatase as a control site was the finding that glucose, which decreased phosphorylase *a* in the whole diaphragm (*14*) and in perfused liver (*17, 18*), also increased phosphorylase phosphatase activity in vitro (*33*).

Cyclic AMP–Dependent Protein Kinase

Cyclic AMP–dependent protein kinase is a component of several of the interconvertible systems discussed here, namely, phosphorylase, glycogen synthetase, and hormone-sensitive lipase. It is, in fact, the point of action in cyclic AMP modulation of these systems, and the suggestion has been made that all the wide variety of cyclic AMP effects may depend on its stimulation of this enzyme (*34*).

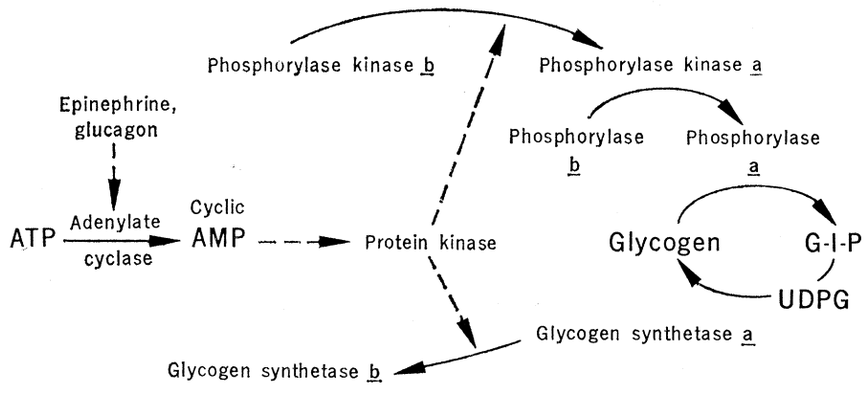

Fig. 1. Epinephrine and glucagon control of glycogen metabolism.

Cyclic AMP–dependent protein kinases have been demonstrated in a wide variety of tissues and species, including multiple forms in some cases [for a recent review, see (*35*)]. A phosphatase that *dephosphorylates* histones and protamine, but not low molecular weight phosphate esters, has also been purified from liver (*36*).

Several recent reports have provided information on the molecular mechanism of the cyclic AMP stimulation of protein kinase. The enzymatically active protein, of molecular weight about 60,000, is closely associated with a regulatory subunit of molecular weight about 80,000. In the associated state the enzyme is inactive. Cyclic AMP binds to and promotes the dissociation of the regulatory subunit, releasing the catalytic moiety in an active form. In addition, at least one of the substrates of protein kinase, histone, also promotes dissociation of the enzyme, yielding the cyclic AMP–independent subunit (*37*).

The Glycogen Synthetase System

It had long been assumed, for lack of a better proposal, that phosphorylase catalyzes glycogen synthesis as well as breakdown, although stimulation of the phosphorylase reaction consistently promotes glycogen breakdown, but not synthesis (*38*). The energetics of the phosphorylase reaction also argue against its involvement in glycogen synthesis (*39*). With the discovery of an independent pathway for the synthesis of glycogen from G-1-P by way of UDPG (*40*) the problem was resolved. Confirming genetic evidence for the operation of a pathway for glycogen synthesis independent of that for its breakdown came from the discovery that, in one of the congenital glycogen storage diseases, high concentrations of glycogen are present in tissues despite absent or low phosphorylase activity (*41*). In these individuals glycogen synthetase activity is normal.

The original elucidation of the pathway was in liver tissue (*40*). Shortly thereafter the pathway was demonstrated in muscle (*42*) and other animal tissues (*43*), and subsequently the same or related pathways have been found in every cell examined where glycogen or starch is present, including microorganisms.

With the existence of separate pathways for the interconversion of G-1-P and glycogen, control of glycogen synthesis and breakdown at these points becomes feasible, indeed indispensable, to avoid energy short-circuiting. Moreover, once the uridyl pathway was selected to provide for carbohydrate storage, control at the glycogen synthetase reaction, rather that at an earlier step in the pathway, became essential in animals to avoid complications with galactose and glucuronic acid formation. In plants, where the analogous reaction is not a branch point (*44*), control is at the previous step (*45*).

Early in the investigations of the glycogen synthetase system it was found that G-6-P stimulated the reaction in tissue extracts (*43*). Larner and his co-workers subsequently established that the degree of G-6-P dependent and G-6-P independent activity was a reflection of the relative amounts of two forms of the enzyme (*46*), which they refer to as the D, or G-6-P dependent form, and the I, or G-6-P independent form, respectively (*47*). While it is well established that tissue extracts exhibit G-6-P dependent and independent glycogen synthetase activities as assayed in vitro, it is not precisely correct to refer to G-6-P dependent and independent enzyme *forms*. Both forms are stimulated by G-6-P, but with different quantitative and, in the case of muscle, qualitative characteristics (*47–49*). The increased activity with G-6-P present is a result of the stimulation of both forms, one considerably more than the other under assay conditions. For this reason we have preferred the more noncommittal terminology, *a* and *b*, to refer to the physiologically active and inactive forms, respectively (*49*), as had been originally employed by the Larner group (*50*).

With the establishment that the two forms were interconvertible by phosphorylation and dephosphorylation reactions analogous to the phosphorylase system, except that the dephosphorylated form of glycogen synthetase is the active one [see (*51*) and (*52*)], it became evident that the existence of interconvertible forms of enzymes with differing activities was not unique to the phosphorylase system, but was a more general phenomenon than had been realized.

Muscle glycogen synthetase. Recently muscle glycogen synthetase has been obtained in a high state of purity and found to undergo incorporation of one phosphate group per subunit, or four per molecule of around 400,000 daltons, during the *a* to *b* conversion (*53*). It is now established that glycogen synthetase kinase is identical with the cyclic AMP–stimulated protein kinase which phosphorylates phosphorylase kinase as well as a number of other proteins (*53*). In this case the link is direct, unlike the phosphorylase system where phosphorylase *b* is not phosphorylated directly by cyclic AMP–dependent protein kinase, but instead another kinase (phosphorylase kinase) intervenes between protein kinase and the metabolic enzyme itself. The result is that a double-pronged regulatory system is provided, in which the cyclic AMP–stimulated protein kinase turns on glycogen degradation by way of the phosphorylase reaction and turns off glycogen synthetase–catalyzed glycogen synthesis (Fig. 1). It is not yet known with equal certainty what relationship exists among the phosphatases that activate glycogen synthetase and inactivate phosphorylase and thus reverse the metabolic flow, although some interesting suggestions have been proposed in this regard (*54, 55*), as is discussed below.

Progress has been made by Piras and his co-workers in elucidating the relevant factors in the modulation of muscle glycogen synthetase activity in vivo. Their results indicate that changes in concentrations of regulatory ligands and interconversion between active and inactive forms both play a role in the regulation of flow through the glycogen synthetase reaction (*56*).

Glycogen itself also appears to be a modulator of the synthetase reaction. When glycogen content and synthetase *a* activity were compared in muscles analyzed at various times after electrical stimulation (*57*) or in hearts perfused with and without oxygen and glucose (*58*), there was an inverse relationship between these components. A possible mechanism for this relationship is suggested by the ability of glycogen at physiological levels to inhibit the phosphatase that forms synthetase *a* (*51, 58*). There is an opposite effect of glycogen on the phosphorylase system, in that glycogen stimulates phosphorylase kinase (*20*), as well as phosphorylase itself (*59*), thus reinforcing the effect on the glycogen synthetase system in regulating its own level.

In addition to its modulation by factors related to muscle cell function per se, muscle glycogen synthetase is also responsive to hormonal signals. Moderate doses of epinephrine led to a reduction of the *a* form of the enzyme in isolated diaphragm (*60, 61*). This effect can presumably be attributed to the cyclic AMP stimulation (*62*) of protein kinase

which converts glycogen synthetase *a* to *b* (*53*). Insulin, on the other hand, produced the reverse effect, that is, an increase in the *a* form of synthetase (*63*), by a mechanism independent of cyclic AMP (*61, 64*).

Liver glycogen synthetase. Liver glycogen synthetase undergoes interconversions between active and inactive forms by what appear to be phosphorylation and dephosphorylation reactions (*65, 66*) as in the case of the muscle system. Homogeneous preparations of synthetase *b* have been obtained from both trout (*67*) and rat (*68*) livers. In both cases activity was distributed among a number of interrelated components separable by centrifugation.

The glycogen synthetase kinase (protein kinase) of liver and muscle are cross-reactive with the muscle and liver synthetases (*69*). Liver glycogen synthetase phosphatase, on the other hand, is reported not to act on muscle synthetase (*65*).

The factors involved in the control of the glycogen synthetase reaction in liver have been discussed by Hers *et al.* (*70*). The *b* form of liver synthetase appears to be inactive at all concentrations of relevant ligands likely to occur in vivo, while the correlation between rates of glycogen synthesis and levels of synthetase *a* is high in a variety of conditions. Thus control of the reaction is concluded to be based entirely on a switching between active and inactive forms by effects either on the kinase or the phosphatase, or both simultaneously.

With the exception of glycogen, which inhibits the synthetase phosphatase reaction and thus its own synthesis, all of the chemical signals to which the interconverting systems are known to respond are external, that is, glucose or hormones. Glucagon and epinephrine reduce the level of active synthetase, as does administered cyclic AMP itself. The effects of these hormones can be attributed to elevations of intracellular cyclic AMP with the consequent stimulation of the kinase that converts synthetase to the inactive form. In addition there is evidence for a decreased activity, after glucagon administration, of the phosphatase which catalyzes the reverse reaction (*71*). Glucose produces the opposite effect, that is, an elevation of synthetase *a* by a mechanism not mediated by cyclic AMP (*17, 18*). It is this response, rather than an increased supply of precursor, that accounts for the glucose-induced stimulation of liver glycogen synthesis.

A glucose effect distinct from these rapid responses has been observed in adrenalectomized animals that have been starved for 48 hours. Under these conditions, where activity of synthetase *a* and synthetase phosphatase has completely disappeared, glucose administration produced a restoration of activity, which required about 1½ hours to appear and which was prevented by cycloheximide but not actinomycin (*72*). Livers from adrenalectomized, starved animals do not demonstrate the rapid response to glucose infusion as those from normal animals do (*18*).

The long-known effect of glucocorticoids in promoting liver glycogen deposition can be attributed, at least in part, to an influence of these hormones on the glycogen synthetase system. Several hours after their administration, an increase in synthetase *a* is evident. This increase is the result of the reappearance of phosphatase activity (*73*). The response of adrenalectomized, starved livers to glucocorticoids is blocked by cycloheximide and actinomycin and thus appears to depend upon induction of an as yet unidentified enzyme (*72*).

Although some of the reports of an insulin-induced increase in liver glycogen synthetase *a* must be discounted because of the concomitant administration of glucose, which produces this effect independently of insulin, it does appear that insulin itself has an analogous action. The insulin response is not mediated by cyclic AMP, but appears to act by effects on the phosphatase reaction.

Glycogen synthetase phosphatase. Before the maximum rate in the synthetase phosphatase reaction in crude systems is attained, there is a time lag that is reduced by the addition of glucose in vitro without affecting the ultimate velocity. One possible explanation of these observations would be the existence of inactive (*b*) and active (*a*) forms of the phosphatase, with the lag reflecting a *b* to *a* conversion under the influence of a glucose-stimulated synthetase phosphatase activating enzyme. The concept of two forms of synthetase phosphatase is supported by observations on diabetic livers, where there appeared to be a form of the phosphatase with greater Mg^{2+} dependency than in insulin-treated animals (*71*).

Recently, an alternative suggestion has been made, based upon the ability of phosphorylase *a* to inhibit the glycogen synthetase phosphatase reaction (*55*), in which it is proposed that the lag represents the period required for removal of phosphorylase *a* by its phosphatase—a reaction known to be glucose stimulated (*33*).

Hormone-Sensitive Lipase

For some time it had been suspected that the so-called "hormone-sensitive" lipase of adipose tissue, which hydrolyzes triglycerides, existed in states of differing activity, and convincing evidence in support of this proposal has recently been adduced. Increased activity of this enzyme is obtained after brief exposure of fat tissue or isolated adipocytes to epinephrine, norepinephrine, glucagon, adrenocorticotropic hormone, thyroid-stimulating hormone, luteinizing hormone, serotonin, or vasopressin (*74–76*). On the other hand, insulin or prostaglandin E_1 has reverse or antagonistic effects (*77, 78*). These hormonally induced changes in lipase activity occur concomitantly with changes in intracellular cyclic AMP (*76, 78*), although the correlation of the cyclic AMP levels with rates of lipolysis is not precise. Glucocorticoids, growth hormone, and thyroid hormone also lead to increases in activity of triglyceride lipase, but by a much slower, actinomycin- and puromycin-sensitive process (*79, 80*).

Incubation of isolated fat tissue or homogenates therefrom under certain conditions leads to a progressive decline in triglyceride lipase activity (*79, 81*), which can then be restored by the addition of ATP and cyclic AMP in vitro (*81, 82*). Activation of highly purified preparations, or preparations in which the endogenous protein kinase activity has been blocked by the addition of protein kinase inhibitor, require in addition the presence of exogenous protein kinase, which can be supplied from rabbit muscle (*83, 84*). Concomitant with the protein kinase–dependent activation of purified lipase there is a parallel incorporation of phosphate from [γ-^{32}P]ATP into the lipase preparation (*85*). However, it is not yet established whether the lipase itself or an intervening component is the direct substrate of cyclic AMP–stimulated protein kinase. The degree of activation achieved thus far by this process has only been about twofold, suggesting either that the *b* form of the enzyme has substantial activity or that the preparations thus far obtained contain substantial amounts of the *a* form.

Other lipase activities are present in

adipose tissue; however, these seem to be specific for di- and monoglycerides and, furthermore, are in considerable excess over the triglyceride lipase activity (*79*). Moreover, there is a correlation between hormonal augmentation of the release of free fatty acids and glycerol and of triglyceride lipase activity, but not that of di- and monoglyceride lipase (*86*). Both of these points indicate that the activity of the triglyceride lipase is the rate-limiting factor in the overall process of fatty acid release. In addition, prior incubation of the tissue with lipolytic hormones yielded enzyme that was not further activated by the protein kinase system (*82, 83*), supporting the conclusion that the in vitro activatable lipase is identical with the hormone-sensitive lipase in vivo. Thus it appears that the interconversion of active and inactive (or less active) forms of this lipase underlies the hormonal control of free fatty acid release from adipose tissue and perhaps from other tissues as well (*74*).

The Pyruvate Dehydrogenase System

Pyruvate lies at a major branch point in energy metabolism. Oxidation by way of the pyruvate dehydrogenase reaction leads to acetyl coenzyme A and thence by way of citrate to carbon dioxide with the production of the major portion of the energy supply of aerobic cells. On the other hand, carboxylation to oxalacetate through the pyruvate carboxylase reaction is the initial step leading to hexose formation in liver and kidney.

The pyruvate dehydrogenase complex, as isolated both from *Escherichia coli* and mammalian mitochondria, is a multimer of high molecular weight consisting of a number of pyruvate dehydrogenase, dihydrolipoyl transacetylase, and dihydrolipoyl dehydrogenase units in a stoichiometric relationship to one another (*87*). Recently Reed and coworkers (*88, 89*) and Wieland and coworkers (*90*) have demonstrated that mammalian pyruvate dehydrogenase exists in active (*a*) and inactive (*b*) forms, interconvertible through phosphorylation by an ATP-dependent kinase and a phosphatase-catalyzed dephosphorylation. Pyruvate dehydrogenase resembles glycogen synthetase and glutamine synthetase (discussed below) in that it is the substituted form which is the inactive one. Since the pyruvate dehydrogenase, transacetylase, and lipoyl dehydogenase activities of the pyruvate dehydrogenase complex can be assayed individually and the components can be resolved, it was possible to show that only the pyruvate dehydrogenase activity was affected by phosphorylation of the complex and that of this component was the site of phosphorylation (*88*). As isolated, the pyruvate dehydrogenase complex contains the kinase firmly bound, and variable amounts of the phosphatase, which more readily disassociates. The kinase, as is the case with the pyruvate dehydrogenase and lipoyl dehydrogenase components, is attached to the transacetylase but has been separated from it (*89*). The control enzymes are not specific in regard to the tissue of origin of the pyruvate dehydrogenase on which they act, although some quantitative differences in rate were observed (*89*).

Cyclic AMP has no effect on the kinase reaction, thus distinguishing this kinase from the cyclic AMP–dependent protein kinase discussed earlier. On the other hand, the cyclic nucleotide in the presence of ATP stimulates the phosphatase-catalyzed reactivation of inactive pyruvate dehydrogenase. Furthermore, phosphatase-containing fractions incubated with [^{32}P]ATP undergo cyclic AMP–dependent phosphorylation (*90*). These findings suggest that the phosphatase itself exists in an active, phosphorylated form and an inactive, nonphosphorylated form. There is no information at present regarding a possible relationship between the phosphorylating enzyme for the phosphatase and the cyclic AMP–dependent protein kinase described above.

There is an evident concomitancy in the actions of cyclic AMP on the phosphorylase and pyruvate dehydrogenase systems, providing an integration of the pathway of carbohydrate oxidation at these two points. Cyclic AMP effects at other critical points in the sequence, particularly on the phosphofructokinase reaction (*91*), also appear to exist.

Pyruvate and ADP inhibit the kinase-catalyzed inactivation of pyruvate dehydrogenase, offering at least the possibility for control of the reaction by these modulators (*89*). The shift in the system from dominance of the kinase (inactivation) reaction at low Mg^{2+} concentration to that of the phosphatase (activation) at high Mg^{2+} has led to the suggestion that the system is controlled by variations in the free Mg^{2+} concentration reciprocal to changes in the ATP concentration (*88*).

In starvation and diabetes, where the concentration of free fatty acids in the serum is elevated and metabolism of fatty acids is favored over that of carbohydrate, the amount of pyruvate dehydrogenase in the *a* form in heart and kidney was greatly reduced (*92*), suggesting a link between this system and the hormone-sensitive lipase discussed above. Nicotinic acid, which reduces the concentration of free fatty acids in the serum, also restored pyruvate dehydrogenase *a* to normal levels. Insulin in fat pads led to a conversion of pyruvate dehydrogenase to the *a* form (*93*), as did fructose in perfused liver (*94*). Thus interconversion of active and inactive forms of pyruvate dehydrogenase is indicated as an underlying mechanism in the regulation of glucose oxidation in these conditions.

The Glutamine Synthetase System

In addition to control of the glutamine synthetase reaction by repression and an extremely complex type of feedback inhibition (*95*), the enzyme from *E. coli* also undergoes interconversions between active and inactive forms. Holzer and his co-workers observed that glutamine synthetase activity was markedly and rapidly reduced upon addition of small quantities of ammonium ion (NH_4^+) to cells, by a process independent of protein turnover (*96*). Furthermore, activity was restored by resuspension of the cells in an NH_4^+-free medium, by a process independent of protein synthesis. Inactivation could be reproduced in a cell-free system in the presence of ATP and Mg^{2+}, plus glutamine and an inactivating enzyme present in cell extracts. The inactive and active species were referred to as *b* and *a* forms, respectively. At about the same time Stadtman and his co-workers reported the isolation of glutamine synthetase preparations of different kinetic properties from *E. coli* cultures grown under different conditions of nitrogen nutrition (*97*). These forms differed from one another by the presence (synthetase II) or absence (synthetase I) of adenylyl groups (*98, 99*) covalently bound to tyrosine residues of the enzyme through a phosphodiester link (*100*). In the fully adenylylated enzyme molecule, the number of adenylyl

residues approached 12 (*98, 101*), corresponding to the 12 subunits of glutamine synthetase. Deadenylylation was originally accomplished with the use of exogenous phosphodiesterase (*98, 101*), but subsequently an endogenous deadenylylating enzyme was demonstrated (*102*). It was soon evident that the active (*a*) and inactive (*b*) forms corresponded to the nonadenylylated (I) and adenylylated (II) forms, respectively. Pyrophosphate has been demonstrated to be the coproduct of the adenylylation reaction (*a* to *b* conversion) (*103*). Contrary to what was surmised earlier, it is now known that the deadenylylation reaction consists of a phosphorolysis rather than a hydrolysis, yielding ADP as a product (*104*).

From the properties of the *b* and *a* forms of the enzyme it is clear that the interconversions constitute an on-off switch for the glutamine synthetase reaction (*105*). In the presence of Mg^{2+}, only the *a* form is active. Furthermore, the *b* form but not the *a* form is subject to feedback inhibition by products of glutamine metabolism (*106, 107*). The relative cellular content of *a* and *b* forms is closely linked to the availability of extracellular NH_4^+ (and thus intracellular glutamine) (*107*).

Control of the interconversion reactions is impressive. Glutamine promotes the adenylylation reaction (*96, 108*) and thereby the accumulation of the inactive *b* form of the synthetase. α-Ketoglutarate, on the other hand, has the reverse effect (*102, 108*). Thus in the presence of a high ratio of glutamine to α-ketoglutarate the glutamine synthetase system is switched off, and a low glutamine to α-ketoglutarate ratio switches it on. Feedback inhibition of glutamine utilization by its end products (*95*) ensures the accumulation of glutamine under conditions of "nitrogen saturation" to provide the signal for the "off" conversion of glutamine synthetase.

The enzymatic components of the adenylylation and deadenylylation system are closely related to one another. One component, referred to as P_I, catalyzes both adenylylation and deadenylylation (*108*). A second component, P_{II}, exists in two forms, which, in the presence of P_I, stimulate the adenylylation reaction (P_{II}-AT form) and the deadenylylation reaction (P_{II}-DA form), respectively. The form P_{II}-AT can be converted to P_{II}-DA by a factor present in the P_I preparations, in a reaction requiring α-ketoglutarate and inhibited by glutamine. There is tentative evidence that a uridyl moiety is incorporated into P_{II} during the conversion. The reaction in the other direction has not yet been demonstrated.

Other Systems

A close relationship between histone phosphorylation and hormonal induction of specific proteins in mammary cells has been proposed (*109*), and the suggestion has been made that phosphorylation and dephosphorylation of histones are involved in selective gene expression (*36, 109*). It should be mentioned that a cyclic GMP–stimulated protein kinase which phosphorylates histones has also been described (*110*). Histones are subject to acetylation and deacetylation reactions as well (*111*).

Somewhat conflicting findings have appeared involving interconversions of *E. coli* RNA polymerase. In one case mammalian protein kinase was reported to activate the polymerase with a concomitant transfer of phosphate from the gamma position of ATP to the sigma component of the enzyme (*112*). In another study the effect of ATP was the reverse, producing an inactivation with a transfer of the adenylyl group to the polymerase in the presence of an endogenous transferase (*113*). Mammalian protein kinase also activates *E. coli* polynucleotide phosphorylase (*114*) in a reaction which, like that with the polymerase (*112*), is at least partially cyclic AMP–dependent.

Phosphorylation of ribosomes has been demonstrated by a number of workers, both in vivo and in vitro (*115, 116*), and a relationship between ribosome function and phosphorylation of ribosomal protein has been proposed (*115*). Thus a number of indications exist of a possible role of covalent interconversions of functional units in the processes of protein synthesis.

Another function for cyclic AMP–stimulated phosphorylation reactions is suggested by the finding that isolated neurotubular subunits are phosphorylated in the presence of ATP by a cyclic AMP–stimulated intrinsic protein kinase (*117*). Added protein kinase isolated from brain further augmented the reaction. A mediation in this way of the cyclic AMP effect on neurotransmitter release and of secretory processes in general is thus a possibility.

Suggestive evidence for the existence of interconvertible forms of several additional systems has appeared (*118*), and the possibility seems likely that other as yet undiscovered protein interconversions may also play a role in the control of metabolic pathways and other physiological processes.

Concluding Remarks

Four separate categories of enzyme control can now be distinguished. Two relate to control of enzyme level, namely, control of enzyme synthesis and control of enzyme degradation, and two are based on regulation of enzyme activity, namely, modulator control and interconversion of active and inactive forms. The last provides an additional control device for modulating the extent and even the direction of flux of a metabolic pathway by manipulating the proportion of a metabolic enzyme that is in the physiologically active as compared to the physiologically inactive state. This mechanism and modulation by ligand binding are in some instances complementary and mutually reinforcing. They have in common rapidity of response and potentiality for direct multisite integrative effects.

The interconversion device, however, confers certain unique capabilities. It allows metabolic reactions to be switched on and off without changes in the intracellular concentration of metabolites. For example, small accumulations of cyclic AMP in liver or muscle cells turn on the phosphorylase reaction and turn off the glycogen synthetase reaction, thereby rapidly reversing the direction of flow of glycogen metabolism. The same chemical signal activates triglyceride lipase in adipocytes with concomitant fatty acid release. The signal in the case of pyruvate dehydrogenase appears to be fatty acids, which switches the cell from carbohydrate to fatty acid catabolism.

All these signals appear as a result of situations external to the responsive cell, which are most often transmitted by way of hormonal mediators. That is to say, the metabolic machinery of the responsive cell in these examples is altered in response to a need elsewhere in the organism, whether that need be to provide circulatory glucose at the expense of the liver stores of glycogen, to support exceptional demands on muscle tissue at the expense of its carbohydrate stores, or to provide metabolizable fuel for organic needs at the

expense of the adipose stores. The effect of these processes is to switch enzymes from a form whose properties are adapted to serve the need for intracellular homeostasis to one which overrides these constraints and serves a more urgent need of the whole organism. This argument is obviously inapplicable to interconvertible systems in unicellular organisms, such as the glutamine synthetase system of *E. coli*. There remains in such cases, however, the evident increased scope for modulator binding and regulation in parallel to the increased number of protein components in the system.

References and Notes

1. G. T. Cori and A. A. Green, *J. Biol. Chem.* **151**, 31 (1943).
2. R. Mankovitz and H. L. Segal, *Biochemistry* **8**, 3757 (1969).
3. W. K. Paik and S. Kim, *Science* **174**, 114 (1971).
4. J. Kraut, in *The Enzymes*, P. D. Boyer, Ed. (Academic Press, New York, 1971), vol. 3, p. 165; D. F. Steiner, *Trans. N.Y. Acad. Sci. Ser. 2* **30**, 60 (1967).
5. M. E. Grant and D. J. Prockop, *N. Engl. J. Med.* **286**, 242 (1972).
6. R. G. MacFarlane *et al.*, *Proc. Roy. Soc. Ser. B* **173**, 259 ff. (1969).
7. E. G. Krebs, A. B. Kent, E. H. Fischer, *J. Biol. Chem.* **231**, 73 (1958).
8. Abbreviations are: G-6-P, glucose 6-phosphate; UDPG, uridine diphosphate glucose; G-1-P, glucose-α-1-phosphate; AMP, adenosine monophosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate; GMP, guanosine monophosphate; P_i, inorganic phosphate.
9. E. H. Fischer, A. Pocker, J. C. Saari, *Essays Biochem.* **6**, 23 (1970); E. H. Fischer, L. M. G. Heilmeyer, Jr., R. H. Haschke, *Curr. Top. Cell. Regul.* **4**, 211 (1971).
10. M. Cornblath, P. J. Randle, A. Parmeggiani, H. E. Morgan, *J. Biol. Chem.* **238**, 1592 (1963).
11. H. E. Morgan and A. Parmeggiani, *ibid.* **239**, 2435 (1964); *ibid.*, p. 2440.
12. E. Helmreich and C. F. Cori, *Advan. Enz. Regul.* **3**, 91 (1965).
13. ———, *Pharmacol. Rev.* **18**, 189 (1966).
14. P. A. Holmes and T. E. Mansour, *Biochim. Biophys. Acta* **156**, 266 (1968).
15. E. W. Sutherland, *Science* **177**, 401 (1972).
16. T. W. Rall and E. W. Sutherland, *J. Biol. Chem.* **232**, 1065 (1958); B. Weintraub, E. J. Sarcione, J. E. Sokal, *Amer. J. Physiol.* **216**, 521 (1969).
17. H. Buschiazzo, J. H. Exton, C. R. Park, *Proc. Nat. Acad. Sci. U.S.A.* **65**, 383 (1970).
18. W. Glinsmann, G. Pauk, E. Hern, *Biochim. Biophys. Acta* **39**, 774 (1970).
19. E. G. Krebs, D. J. Graves, E. H. Fischer, *J. Biol. Chem.* **234**, 2867 (1959).
20. E. G. Krebs, D. S. Love, G. E. Bratvold, K. A. Trayser, W. L. Meyer, E. H. Fischer, *Biochemistry* **3**, 1022 (1964).
21. G. A. Riley, *Fed. Proc.* **22**, 258 (1963).
22. R. J. DeLange, R. G. Kemp, W. D. Riley, R. A. Cooper, E. G. Krebs, *J. Biol. Chem.* **243**, 2200 (1968).
23. J. T. Stull, C. O. Brostrom, E. G. Krebs, *ibid.* **247**, 5272 (1972).
24. D. A. Walsh, J. P. Perkins, C. O. Brostrom, E. S. Ho, E. G. Krebs, *ibid.* **246**, 1968 (1971).
25. M. A. Brostrom, E. M. Reimann, D. A. Walsh, E. G. Krebs, *Advan. Enz. Regul.* **8**, 191 (1970).
26. W. D. Riley, R. J. DeLange, G. E. Bratvold, E. G. Krebs, *J. Biol. Chem.* **243**, 2209 (1968).
27. J. B. Posner, R. Stern, E. G. Krebs, *ibid.* **240**, 982 (1965); D. H. Namm and S. E. Mayer, *Mol. Pharmacol.* **4**, 61 (1968).
28. G. I. Drummond, J. B. Harwood, C. A. Powell, *J. Biol. Chem.* **244**, 4235 (1969).
29. S. E. Mayer, D. H. Namm, J. P. Hickenbottom, *Advan. Enz. Regul.* **8**, 205 (1970).
30. L. M. G. Heilmeyer, Jr., F. Meyer, R. H. Haschke, E. H. Fischer, *J. Biol. Chem.* **245**, 6649 (1970).
31. C. O. Brostrom, F. L. Hünkeler, E. G. Krebs, *ibid.* **246**, 1961 (1971).
32. W. Merlevede and G. A. Riley, *ibid.* **241**, 3517 (1966).
33. P. A. Holmes and T. E. Mansour, *Biochim. Biophys. Acta* **156**, 275 (1968); W. Stalmans, H. De Wulf, B. Lederer, H. G. Hers, *Eur. J. Biochem.* **15**, 9 (1970).
34. J. F. Kuo and P. Greengard, *J. Biol. Chem.* **244**, 3417 (1969).
35. E. G. Krebs, *Curr. Top. Cell. Regul.* **5**, 99 (1972).
36. M. H. Meisler and T. A. Langan, *J. Biol. Chem.* **244**, 4961 (1969).
37. E. Miyamoto, G. L. Petzold, J. S. Harris, P. Greengard, *Biochem. Biophys. Res. Commun.* **44**, 305 (1971).
38. P. W. Robbins, R. R. Traut, F. Lipmann, *Proc. Nat. Acad. Sci. U.S.A.* **45**, 6 (1959).
39. M. Cohn, *Enzymes* **5**, 179 (1961).
40. L. F. Leloir and C. E. Cardini, *J. Amer. Chem. Soc.* **79**, 6340 (1957).
41. W. F. H. M. Mommaerts, B. Illingworth, C. M. Pearson, R. J. Guillory, K. Seroydarian, *Proc. Nat. Acad. Sci. U.S.A.* **45**, 791 (1959).
42. C. Villar-Palasi and J. Larner, *Biochim. Biophys. Acta* **30**, 449 (1958).
43. L. F. Leloir, J. M. Olavarria, S. H. Goldemberg, H. Carminatta, *Arch. Biochem. Biophys.* **81**, 508 (1959).
44. R. M. Roberts, *J. Biol. Chem.* **246**, 4995 (1971).
45. H. P. Ghosh and J. Preiss, *ibid.* **241**, 4491 (1966).
46. C. Villar-Palasi and J. Larner, *Arch. Biochem. Biophys.* **94**, 436 (1961).
47. M. Rosell-Pérez, C. Villar-Palasi, J. Larner, *Biochemistry* **1**, 763 (1962).
48. M. Rosell-Pérez and J. Larner, *ibid.* **3**, 75 (1964).
49. H. J. Mersmann and H. L. Segal, *Proc. Nat. Acad. Sci. U.S.A.* **58**, 1688 (1967).
50. C. Villar-Palasi and J. Larner, *Abstr. 138th Amer. Chem. Soc. Mtg.* (1960), 78C.
51. J. Larner, *Trans. N.Y. Acad. Sci. Ser. 2* **29**, 192 (1966).
52. ——— and C. Villar-Palasi, *Curr. Top. Cell. Regul.* **3**, 195 (1971).
53. T. R. Soderling, J. P. Hickenbottom, E. M. Reimann, F. L. Hunkeler, D. A. Walsh, E. G. Krebs, *J. Biol. Chem.* **245**, 6317 (1970).
54. H. De Wulf, W. Stalmans, H. G. Hers, *Eur. J. Biochem.* **15**, 1 (1970).
55. W. Stalmans, H. De Wulf, H. G. Hers, *ibid.* **18**, 582 (1971).
56. R. Piras, L. B. Rothman, E. Cabib, *Biochem. Biophys. Res. Commun.* **28**, 54 (1967); *Biochemistry* **7**, 56 (1968); R. Piras and R. Staneloni, *ibid.* **8**, 2153 (1969).
57. W. H. Danforth, *J. Biol. Chem.* **240**, 588 (1965).
58. F. Huijing, F. Q. Nuttall, C. Villar-Palasi, J. Larner, *Biochim. Biophys. Acta* **177**, 204 (1969).
59. B. Metzger, E. Helmreich, L. Glaser, *Proc. Nat. Acad. Sci. U.S.A.* **57**, 994 (1967).
60. J. W. Craig and J. Larner, *Nature* **202**, 971 (1964).
61. J. W. Craig, T. W. Rall, J. Larner, *Biochim. Biophys. Acta* **177**, 213 (1969).
62. M. Rosell-Pérez and J. Larner, *Biochemistry* **3**, 81 (1964).
63. C. Villar-Palasi and J. Larner, *Biochim. Biophys. Acta* **39**, 171 (1960).
64. J. Larner, *Diabetes* **21** (Suppl. 2), 428 (1972).
65. S. Hizukuri and J. Larner, *Biochemistry* **3**, 1783 (1964).
66. H. De Wulf and H. G. Hers, *Eur. J. Biochem.* **6**, 552 (1968).
67. D. C. Lin, H. L. Segal, E. J. Massaro, *Biochemistry* **11**, 4466 (1972).
68. D. C. Lin and H. L. Segal, in preparation.
69. A. T. Yip and J. Larner, *Physiol. Chem. Phys.* **1**, 383 (1969).
70. H. G. Hers, H. De Wulf, W. Stalmans, G. Van den Berghe, *Advan. Enz. Regul.* **8**, 171 (1970); H. G. Hers, H. De Wulf, W. Stalmans, *Fed. Eur. Biochem. Soc. Lett.* **12**, 73 (1970).
71. J. S. Bishop, *Biochim. Biophys. Acta* **208**, 208 (1970).
72. K. Gruhner and H. L. Segal, *ibid.* **222**, 508 (1970).
73. H. J. Mersmann and H. L. Segal, *J. Biol. Chem.* **244**, 1701 (1969).
74. M. Vaughan, *Pharmacol. Rev.* **18**, 215 (1966).
75. R. W. Butcher and E. W. Sutherland, *Ann. N.Y. Acad. Sci.* **139**, 849 (1967).
76. R. W. Butcher, C. E. Baird, E. W. Smith, *J. Biol. Chem.* **243**, 1705 (1968).
77. R. W. Butcher and C. E. Baird, *ibid.*, p. 1713.
78. V. C. Manganiello, F. Murad, M. Vaughan, *ibid.* **246**, 2195 (1971).
79. D. Steinberg, *Pharmacol. Rev.* **18**, 217 (1966).
80. J. N. Fain, *Ann. N.Y. Acad. Sci.* **139**, 879 (1967).
81. M. A. Rizack, *J. Biol. Chem.* **239**, 392 (1964).
82. S.-C. Tsai, P. Belfrage, M. Vaughan, *J. Lipid Res.* **11**, 466 (1970).
83. J. K. Huttunen, D. Steinberg, S. E. Mayer, *Proc. Nat. Acad. Sci. U.S.A.* **67**, 290 (1970).
84. J. D. Corbin, E. M. Reimann, D. A. Walsh, E. G. Krebs, *J. Biol. Chem.* **245**, 4849 (1970).
85. J. K. Huttunen, D. Steinberg, S. E. Mayer, *Biochem. Biophys. Res. Commun.* **41**, 1350 (1970).
86. M. Vaughan, *Ann. N.Y. Acad. Sci.* **139**, 841 (1967).
87. L. J. Reed, *Curr. Top. Cell. Regul.* **1**, 233 (1969).
88. T. C. Linn, F. H. Pettit, L. J. Reed, *Proc. Nat. Acad. Sci. U.S.A.* **62**, 234 (1969).
89. T. C. Linn, F. H. Pettit, F. Hucho, L. J. Reed, *ibid.* **64**, 227 (1969).
90. O. Wieland and E. Siess, *ibid.* **65**, 947 (1970).
91. T. E. Mansour, *J. Biol. Chem.* **247**, 6059 (1972).
92. O. Wieland, E. Siess, F. H. Schulze-Wethmar, H. J. v. Funcke, B. Winton, *Arch. Biochem. Biophys.* **143**, 593 (1971).
93. L. Weiss, G. Löffler, A. Schirmann, O. Wieland, *Fed. Eur. Biochem. Soc. Lett.* **15**, 229 (1971); R. M. Denton, H. G. Coove, B. R. Martin, P. J. Randle, *Nature New Biol.* **231**, 115 (1971).
94. H. D. Soling and G. Bernhard, *Fed. Eur. Biochem. Soc. Lett.* **13**, 201 (1971).
95. E. R. Stadtman, B. M. Shapiro, H. S. Kingdon, C. A. Woolfolk, J. S. Hubbard, *Advan. Enz. Regul.* **6**, 257 (1968).
96. H. Holzer, D. Mecke, K. Wulff, K. Liess, L. Heilmeyer, Jr., *ibid.* **5**, 211 (1967).
97. H. S. Kingdon and E. R. Stadtman, *J. Bacteriol.* **94**, 949 (1967).
98. B. M. Shapiro, H. S. Kingdon, E. R. Stadtman, *Proc. Nat. Acad. Sci. U.S.A.* **58**, 642 (1967).
99. K. Wulff, D. Mecke, H. Holzer, *Biochem. Biophys. Res. Commun.* **28**, 740 (1967).
100. R. L. Heinrikson and H. S. Kingdon, *J. Biol. Chem.* **246**, 1099 (1971).
101. H. S. Kingdon, B. M. Shapiro, E. R. Stadtman, *Proc. Nat. Acad. Sci. U.S.A.* **58**, 1703 (1967).
102. B. M. Shapiro and E. R. Stadtman, *Biochem. Biophys. Res. Commun.* **30**, 32 (1968).
103. C. P. Heinrich, F. A. Battig, M. Mantel, H. Holzer, *Arch. Mikrobiol.* **73**, 104 (1970).
104. W. B. Anderson and E. R. Stadtman, *Biochem. Biophys. Res. Commun.* **41**, 704 (1970).
105. E. R. Stadtman, A. Ginsburg, J. E. Ciardi, J. Yeh, S. B. Hennig, B. M. Shapiro, *Advan. Enz. Regul.* **8**, 99 (1970).
106. H. S. Kingdon and E. R. Stadtman, *Biochem. Biophys. Res. Commun.* **27**, 470 (1967).
107. H. Holzer, H. Schutt, Z. Masek, D. Mecke, *Proc. Nat. Acad. Sci. U.S.A.* **60**, 721 (1968).
108. M. S. Brown, A. Segal, E. R. Stadtman, *ibid.* **68**, 2949 (1971).
109. R. W. Turkington and M. Riddle, *J. Biol. Chem.* **244**, 6040 (1969).
110. J. F. Kuo, J. Sanes, P. Greengard, *Fed. Proc.* **29**, 601 (1970).
111. W. F. Marzluff, Jr., and K. S. McCarty, *J. Biol. Chem.* **245**, 5635 (1970).
112. O. J. Martelo, S. L. C. Woo, E. M. Reimann, E. W. Davie, *Biochemistry* **9**, 4807 (1970).
113. C. A. Chelala, L. Hirschbein, H. N. Torres, *Proc. Nat. Acad. Sci. U.S.A.* **68**, 152 (1971).
114. M. N. Thang and F. Meyer, *Fed. Eur. Biochem. Soc. Lett.* **13**, 345 (1971).
115. D. Kabat, *Biochemistry* **9**, 4160 (1970).
116. J. A. Traugh and R. R. Trout, *ibid.* **11**, 2503 (1972).
117. D. B. P. Goodman, H. Rasmussen, F. Di Bella, C. E. Guthrow, Jr., *Proc. Nat. Acad. Sci. U.S.A.* **67**, 652 (1970).
118. H. Holzer and W. Duntze, *Annu. Rev. Biochem.* **40**, 345 (1971).

119. This work was supported by a grant from National Institutes of Health (AM-08873).