THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 262, No. 10, Issue of April 5, pp. 4460-4471, 1987
Printed in U.S.A.

Energy Expenditure in the Control of Biochemical Systems by Covalent Modification*

(Received for publication, July 24, 1986)

Albert Goldbeter‡ and Daniel E. Koshland, Jr.§

From the ‡Faculté des Sciences, Université Libre de Bruxelles, Campus Plaine, C. P. 231, B-1050 Brussels, Belgium and the §Department of Biochemistry, University of California, Berkeley, California 94720

Regulation by reversible, covalent modification of proteins requires a continuous expenditure of energy, even in a steady-state situation. The cost of this energy drain is evaluated for the case of an effector controlling the modifying enzyme and an effector controlling the demodifying enzyme and for the case of dual control in which an effector activates one of these enzymes and inhibits the other. Energy consumption is determined when the converter enzymes are functioning in the first-order and zero-order domains. The profile of energy expenditure *versus* fractional protein modification at steady state varies both as a function of the mechanism of control of the converter enzymes and of the kinetic domain in which they operate. This theory allows one to predict the strategies that would minimize energy costs. Dual control appears to provide maximum sensitivity with minimal energy expenditure. The analysis is applied to two experimental systems. Comparison of ATP turnover rates with rates for individual modification enzymes in living systems shows that a significant fraction of the total energy expenditure of an organism is required for the large number of reactions which involve covalent modification of proteins. It is concluded that there will be selection pressure for energy-efficient control of covalent regulation.

Protein covalent modification is one of the central mechanisms for control of biological systems. Fascinating features of this process are its reversibility and its ubiquity. The variety of cellular processes which are controlled by covalent modification is well known. Many enzymes are activated or inactivated through covalent modification (1-6). It has been estimated that as much as one out of six proteins in living systems is reversibly phosphorylated (7, 8). Irreversible covalent modifications also occur, as in the blood coagulation proteolytic cascade (9, 10); but such changes are not useful for controlling systems which must be switched on and off as changing conditions occur during the lifetime of a cell.

The reversibility and ubiquity of covalent modification pose a serious problem for the cell. Maintenance of a steady-state level of modified protein inevitably involves a continuous expenditure of energy. The fact that there are so many systems controlled by covalent modification raises the possibility that even if any one system utilizes a minor amount of energy, the accumulated consumption in many systems could bring a major depletion of the cell's energy resources. Some aspects of energy utilization in metabolic futile cycles have been considered, notably with respect to their possible role in heat generation (11). In relation to covalent modification, Shacter *et al.* (12, 13) have considered the energy utilization in a reversible phosphorylation system using a synthetic nonapeptide substrate. They obtained a linear relationship between ATP expenditure at steady state and the fraction of phosphorylated nonapeptide.

In previous publications (14-16), we analyzed the sensitivity properties of a biological system controlled by covalent modification and concluded that such a system becomes ultrasensitive when the converter enzymes operate in the zero-order region, as compared to Michaelis-Menten-type sensitivity when the kinetics of the modifying enzymes is in the first-order region. It seems therefore worthwhile to evaluate the energy of covalent modification systems operating in regions of high sensitivity and low sensitivity, to determine the extent of the drain on the cell's total energy of such mechanisms of regulation, and to understand the strategies that cells utilize to minimize energy loss.

In the following, we derive the theoretical equations which govern energy expenditure in reversible covalent modification. Different modes of control of the modifying enzymes are considered. This analysis shows that the energy expenditure associated with the maintenance of a steady-state level of protein modification depends both on the mechanism of control of the converter enzymes and on the kinetic domain in which they operate. We apply the theory to two experimental systems and discuss the general implications of these findings for cell metabolism.

THEORY

Steady-state Level of Protein Modification—In a monocyclic covalent modification cascade (Equation 1) in which a protein W is modified by enzyme E_1 into the form W*,

$$\mathrm{W} \underset{E_2}{\overset{E_1}{\rightleftharpoons}} \mathrm{W}^* \qquad (1)$$

the latter being demodified by enzyme E_2, the fraction of modified protein at steady state is a function of the effective maximum rates V_1 and V_2 (*i.e.* the maximum rates of E_1 and E_2 at a given value of their controlling effector J) and of the reduced Michaelis constants K_1 and K_2 of the two modifying enzymes ($K_1 = K_{m1}/\mathrm{W}_T$, $K_2 = K_{m2}/\mathrm{W}_T$, where W_T denotes the total concentration of the protein substrate). The expression relating this fraction, denoted W*, to the above-mentioned parameters of enzymes E_1 and E_2 is (14, 16) as follows.

* This work was supported by grants from the National Institutes of Health and the National Science Foundation and North Atlantic Treaty Organization Research Grant 085/82. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

$$W^* = \frac{\left\{\left(\frac{V_1}{V_2} - 1\right) - K_2\left(\frac{K_1}{K_2} + \frac{V_1}{V_2}\right) + \left(\left[\frac{V_1}{V_2} - 1 - K_2\left(\frac{K_1}{K_2} + \frac{V_1}{V_2}\right)\right]^2 + 4K_2\left(\frac{V_1}{V_2} - 1\right)\left(\frac{V_1}{V_2}\right)\right)^{1/2}\right\}}{2\left(\frac{V_1}{V_2} - 1\right)} \quad (2)$$

In the following, we shall use the symbol W* for denoting the mole fraction of protein modified at steady state. If occasionally W* refers to the modified protein as molecular species, it will be clear from the context. It is important to note that W* at steady state depends only on the ratio V_1/V_2 and not on the absolute values of V_1 and V_2 which govern only the rate of evolution toward the steady state. Thus, a larger fraction of modified protein can be obtained by increasing V_1, decreasing V_2, or by combination of both actions.

We have previously shown (14) that W* varies in an extremely steep manner upon increasing V_1/V_2, provided that K_1 and/or K_2 is much smaller than unity, that is, when the total amount of protein substrate exceeds the Michaelis constants of enzymes E_1 and/or E_2 (see Fig. 1). This increased sensitivity occurs when one of the modification enzymes operates in the zero-order kinetic domain, hence, the name of "zero-order ultrasensitivity" given to this phenomenon (14–16). Experimental support for the theory has been obtained both with a synthetic peptide (12) and with *in vivo* systems (17, 18).

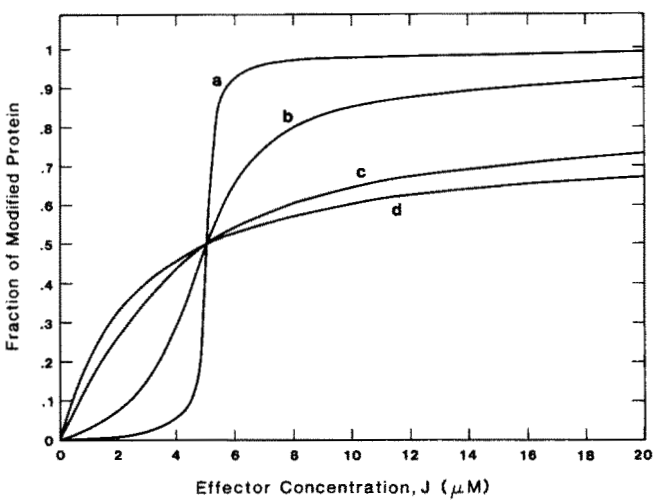

FIG. 1. **Zero-order ultrasensitivity in covalent modification.** The fraction of protein modified at steady state, W*, is plotted according to Equation 2 as a function of the concentration of an effector J which activates enzyme E_1 as in mechanism I in Table I. Then the ratio of maximum effective modification rates appearing in Equation 2 is $V_1/V_2 = (V_{M1}/V_2)J/(K_{J1} + J)$. Curves are established for $(V_{M1}/V_2) = 3$, and $K_{J1} = 10$ μM, for increasing values of the reduced Michaelis constants $K_1 = K_2 = 10^{-2}$ (*curve a*), 10^{-1} (*curve b*), 1 (*curve c*), and 10 (*curve d*). When $K_{m1} = K_{m2} = 1$ μM, these values correspond to a total target protein concentration, W_T, of 100, 10, 1, and 0.1 μM, respectively. The steepness of the transition curve increases as the values of K_1 and K_2 decrease below unity. This phenomenon, previously quantified and referred to as zero-order ultrasensitivity (14–16), is shown here on a nonlogarithmic scale to make clear the transition from sigmoidal to hyperbolic as the values of K_1 and K_2 rise above unity. For values of J yielding a fractional modification smaller than 0.5, a 10–100-fold increase in the target protein concentration produces a significant decrease in the value of W* at steady state. Such a phenomenon accounts for the behavior of the isocitrate dehydrogenase system in the absence of the effector 3-PG in *E. coli* (see "Isocitrate Dehydrogenase Phosphorylation in *E. coli*" and Fig. 7).

Energy Consumption at Steady State in the Covalent Modification Cycle—During the transient approach to steady state, the energy expenditure associated with covalent modification is governed by the activity of the enzyme catalyzing the ATP-requiring modification step. At steady state, to determine the energy consumption, v_{ATP}, we note that the rate of protein modification is equal to the rate of demodification. The value for this quantity is then expressed in Equation 3.

$$v_{ATP} = \frac{V_1 W}{K_1 + W} = \frac{V_2 W^*}{K_2 + W^*} \quad (3)$$

Equation 3 is obtained assuming that both enzymes E_1 and E_2 operate in a Michaelian manner. In case of cooperative kinetics, the rate laws have to be modified, but such changes do not affect significantly the results reported below.

As in a previous publication (16), we shall consider three different mechanisms for the control of E_1 and E_2 by an effector J. These mechanisms are listed in Table I, together with the expressions for the maximum rates of the converter enzymes as a function of J and the expressions for v_{ATP} as a function of W*. In mechanism I, J activates E_1. This mechanism corresponds, for example, to the activation of protein kinase by cAMP. In mechanism II, J inhibits E_2 in a noncompetitive manner by decreasing the maximum rate of the enzyme without affecting its K_m. In mechanism III, J activates E_1 and inhibits E_2 in a noncompetitive manner.

Although different effectors can control the two modification enzymes, the situation in which a single effector regulates directly or indirectly both the kinase and the phosphatase is relatively common. Such is the case for cAMP in the glycogen metabolic cascade (19–21) and for 3-phosphoglycerate in the control of isocitrate dehydrogenase by phosphorylation-dephosphorylation in *Escherichia coli* (17).

Since the maximum rate of E_2 is independent of J in mechanism I and the maximum rate of E_1 is independent of J in mechanism II, the simplest form of the rate of energy expenditure in these two mechanisms is obtained as a function of V_2 and V_1, respectively (see Table I, Equations T1 and T2).

To obtain the rate of energy expenditure in mechanism III where both V_1 and V_2 depend on J, we make use of Equation 4.

$$\frac{V_1}{V_2} = \frac{W^*(1 - W^* + K_1)}{(1 - W^*)(W^* + K_2)} \quad (4)$$

This expression (14) gives the ratio (V_1/V_2) needed for maintaining a given W* level at steady state. Note that neither V_1 nor V_2 is constant in this mechanism. From this equation and from the expressions of V_1 and V_2 listed in Table I, we may extract the value of J corresponding to this particular value of W* in mechanism III. When K_{J1} does not equal K_{J2}, this procedure yields a second-degree equation for J which admits a unique positive value. This value is then inserted into V_1 and V_2, and the rate of energy expenditure is determined according to Equation 3. In the simple case, $K_{J1} = K_{J2}$, this procedure yields a relatively compact expression (Equation T3 in Table I) which permits one to pursue a more detailed analytical treatment. Without loss of generality, we shall therefore use for illustrative purposes this situation in which the half-maximum effects on the control of E_1 and E_2 by J occur at the same effector concentration. A case in which K_{J1} does not equal K_{J2} is considered under "Isocitrate Dehydrogenase Phosphorylation in *E. coli*."

Energy Consumption as a Function of the Fraction of Substrate Protein Modified at Steady State—We are now in a position to determine the variation of energy consumption at

TABLE I

Rate of energy expenditure at steady state, as a function of W, for three mechanisms for the control of the covalent modification cycle*

In mechanism I, effector J activates enzyme E_1; in mechanism II, J inhibits E_2; in mechanism III, J activates E_1 and inhibits E_2. K_{J1} is the activation constant for E_1; K_{J2} is the inhibition constant for E_2. V_{M1} denotes the maximum rate of E_1 at saturation by J; V_{M2} is the maximum rate of E_2 in the absence of J. The constants K_1 and K_2 are defined as $K_1 = K_{m1}/W_T$, $K_2 = K_{m2}/W_T$, where K_{m1} and K_{m2} are the Michaelis constants of E_1 and E_2 and W_T is the total amount of target protein. The fraction of modified protein is defined as $W^* = [W^*]/W_T$. Similarly, the fraction of unmodified protein is defined as $W = [W]/W_T$. Equations T1–T3 yield the rate of energy expenditure as a function of the fraction of protein modified at steady state in the three mechanisms for the control of covalent modification. The rate of ATP expenditure in mechanism III is obtained by calculating from Equation 4 and from the expressions for V_1 and V_2 the value of J required for the V_1/V_2 ratio, yielding the particular value of W*. This value, J_{W^*}, is then used for calculating V_1 or V_2 and the rate of ATP consumption at steady state as a function of W*. A similar procedure is used for determining the steady-state rate of ATP consumption in the isocitrate dehydrogenase system considered under "Application to Experimental Systems." In case W* is varied directly by changes in the concentration of enzymes E_1 or E_2, Equations T1 or T2 should be used, respectively.

Mechanism of control of the converter enzymes by effector J	Maximum rate of E_1	Maximum rate of E_2	Rate of energy expenditure at steady state as a function of W*	
Mechanism I	$V_1 = V_{M1}\left(\frac{J}{K_{J1} + J}\right)$	V_2 [a]	$v_{ATP} = V_2\left(\frac{W^*}{K_2 + W^*}\right)$	(T1)
Mechanism II	V_1 [a]	$V_2 = V_{M2}\left(\frac{K_{J2}}{K_{J2} + J}\right)$	$v_{ATP} = V_1\left(\frac{1 - W^*}{K_1 + 1 - W^*}\right)$	(T2)
Mechanism III	$V_1 = V_{M1}\left(\frac{J}{K_{J1} + J}\right)$	$V_2 = V_{M2}\left(\frac{K_{J2}}{K_{J2} + J}\right)$	$v_{ATP} = \frac{V_{M1}V_{M2}W^*(1 - W^*)}{[V_{M1}(1 - W^*)(W^* + K_2) + V_{M2}W^*(1 - W^* + K_1)]}$	(T3) [b]

[a] Independent of J.
[b] Obtained for $K_{J1} = K_{J2}$.

steady state as a function of the fraction of modified protein. In each of the three control mechanisms described above, we shall consider that E_1 and E_2 operate in either the first-order or zero-order kinetic domain.

The variation of the rate of ATP consumption in mechanisms I–III is shown in Fig. 2 in the case $K_1 = K_2 = 0.1$. These values produce moderate ultrasensitivity (see Fig. 1). The results show that the energy expenditure relation to the amount of modified protein markedly depends on the manner in which the enzymes of the covalent modification system are controlled by the effector J. When the ratio V_1/V_2 which controls the steady-state value of the fraction of modified protein is increased through an increase in V_1 at constant V_2 as in mechanism I, the energy consumption increases with W* in a hyperbolic manner and saturates to a constant level when W* approaches unity. When V_1/V_2 is increased as in mechanism II (through a decrease in V_2 at constant V_1), the energy consumption rises in a hyperbolic manner when the fraction of modified protein decreases from unity to zero. Finally, when the ratio of modification rates is increased through a concomitant increase in V_1 and decrease in V_2 as in mechanism III, the dependence of energy consumption on the fraction of modified protein is bell-shaped; the rate of ATP expended at steady state rises from zero, passes through a maximum, and returns to zero as W* goes from zero to unity.

In the first-order domain (Fig. 3), the energy consumption in mechanism III still possesses a bell shape, although its dependence on W* is much smoother than in Fig. 2. The most striking difference brought about by first-order kinetics relates, however, to mechanisms I and II. In both cases, the curves have changed from hyperbolic to linear.

Explanation of the Dependence of Energy Consumption on the Mode of Control of Modification Enzymes—The results of Figs. 2 and 3 can be explained by examination of the equations which yield the rate of energy consumption at steady state, according to the mechanism of control of the modification enzymes.

Equation T1 (see Table I) for mechanism I shows that v_{ATP} varies in a hyperbolic manner with W* and reaches the constant value $V_2/(K_2 + 1)$ as W* approaches unity. Given that the half-maximum level of energy consumption is reached for $W^* = K_2/(1 + 2K_2)$, the hyperbolic curve becomes steeper and steeper at low W* values as K_2 decreases below unity, *i.e.* when the demodifying enzyme enters the zero-order kinetic domain. When $K_2 \gg 1$, the enzyme E_2 operates in the first-order domain and Equation T1 indicates a linear dependence of v_{ATP} on W* with a slope V_2/K_2, as shown by the corresponding *curve I* in Fig. 3.

In mechanism II, v_{ATP} is at the maximum value $V_1/(K_1 + 1)$ when $W^* = 0$ and decreases to zero in a hyperbolic manner as W* goes to unity. The half-maximum energy consumption is reached for $W^* = (1 + K_1)/(1 + 2K_1)$. Thus, the hyperbola becomes steeper and steeper near $W^* = 1$ when K_1 decreases below unity, *i.e.* when enzyme E_1 progressively enters the zero-order domain. In contrast, when E_1 operates in the first-order domain, Equation T2 (see Table I) shows that v_{ATP} decreases linearly from V_1/K_1 to zero, with a slope $-V_1/K_1$, upon increasing W*.

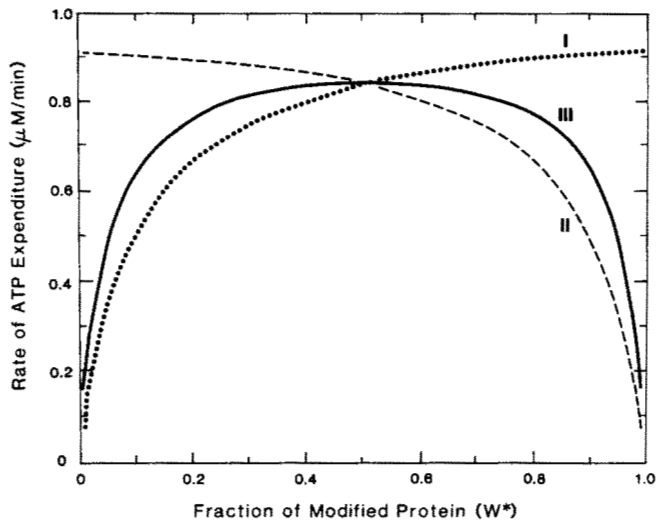

FIG. 2. **Energy expenditure in covalent modification.** The rate of ATP consumption at steady state is shown as a function of the fraction of modified protein for three different mechanisms for the control of the converter enzymes. *Curves I–III* are obtained for the corresponding mechanisms listed in Table I and generated according to Equations T1–T3. Parameter values are $K_1 = K_2 = 0.1$, $K_{J1} = K_{J2} = 1$ μM, $V_2 = 1$ μM/min for *curve I*, $V_1 = 1$ μM/min for *curve II*, and $V_{M1} = V_{M2} = 2$ μM/min for *curve III*. These values of modification rates are taken for illustrative purposes; values larger by several orders of magnitude are observed experimentally for cAMP-dependent protein kinase and glycogen phosphorylase (see "Discussion"). The theoretical curves then remain unchanged, save for a scaling factor. The value 0.1 for K_1 and K_2 yields moderate zero-order ultrasensitivity, as indicated in Fig. 1, *curve b*, for the covalent modification corresponding to mechanism I.

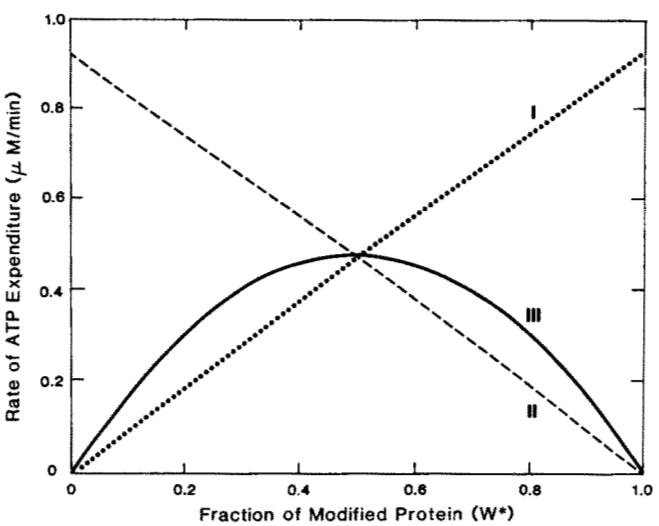

FIG. 3. **Energy expenditure at steady state in covalent modification as a function of fractional modification when the converter enzymes operate in the first-order kinetic domain.** The three curves are obtained as for Fig. 2, with $K_1 = K_2 = 10$, *i.e.* for a total concentration of protein substrate 100-fold smaller than in Fig. 2.

What is the origin of the bell-shaped dependence of energy consumption on the amount of modified protein when V_1/V_2 is changed according to mechanism III? To quantify this result further, we have obtained the analytical expression for the fraction of modified protein corresponding to the maximum energy consumption at steady state, W^*_M. By setting the derivative of v_{ATP} (Equation T3, see Table I) equal to zero, we get the following.

$$W^*_M = \frac{1}{1 + (K_1 V_{M2}/K_2 V_{M1})^{1/2}} \quad (5)$$

The corresponding maximum rate of energy consumption is given by Equation 6.

$$(v_{ATP})_M = \frac{V_{M1} V_{M2}}{V_{M1}(1 + K_2) + V_{M2}(1 + K_1) + 2(K_1 K_2 V_{M1} V_{M2})^{1/2}} \quad (6)$$

For the particular symmetric case $K_1 = K_2$, $V_{M1} = V_{M2}$ considered in Figs. 2 and 3, these relations yield the following simple expressions.

$$W^*_M = 0.5; \qquad (v_{ATP})_M = \frac{V_{M1}}{2(1 + 2K_1)} \quad (7)$$

Both below and above W^*_M, the energy consumption decreases and goes to vanishingly small values as W* tends toward zero or unity. This occurs because the ATP consumption, which is equal to both $V_1 W/(K_1 + W)$ and $V_2 W^*/(K_2 + W^*)$, goes to zero when W or W* approaches unity.

This behavior at both low and high values of the fraction of modified protein has to be contrasted with the behavior of the modification system when E_1 and E_2 are controlled according to mechanisms I or II. In mechanism I, v_{ATP} reaches a maximum value when W* goes to unity. As the amount of unmodified protein W then goes to zero, the energy consumption, which is also equal at steady state to $V_1 W/(K_1 + W)$, at first view should vanish! This contradiction is only apparent, however. As V_2 is constant, W cannot go to zero (nor can W* go totally to unity) unless the maximum rate of E_1, *i.e.* V_{M1} (see Table I), goes to infinity. A similar explanation holds for mechanism II where energy consumption reaches a constant level as W* approaches zero.

Energy Expenditure when One of the Modification Enzymes Operates in the First-order Domain and the Other in the Zero-order Domain—In view of the above-mentioned dependence of energy expenditure on kinetics (compare Figs. 2 and 3), the question arises as to how the energy consumption at steady state depends on the fraction of modified protein when one of the modifying enzymes operates in the first-order region, whereas the other possesses zero-order kinetics. For definiteness, let us assume that E_1 operates with zero-order kinetics ($K_1 \ll 1$), whereas E_2 operates in the first-order domain ($K_2 \gg 1$).

In mechanism I, as enzyme E_2 operates in the first-order domain, Equation T1 dictates a linear dependence of the energy consumption on the fraction of protein modified at steady state. The resulting curve should resemble *curve I* of Fig. 3.

When control mechanism II applies, Equation T2 shows that the form of the energy consumption curve is solely governed by the kinetics of E_1. As this kinetics is zero-order in the case considered, the curve should resemble *curve II* of Fig. 2.

In the case of mechanism III, the situation is more complex as Equation T3 governs the dependence of energy consumption on W*. For the illustrative case where $K_1 = 0.1$, $K_2 = 10$, this equation reduces to the following approximate expression.

$$v_{ATP} = \frac{V_{M1} V_{M2} W^*}{V_{M1} K_2 + V_{M2} W^*} \quad (8)$$

If $V_{M1} > V_{M2}$, this equation takes the linear form $v_{ATP} = (V_{M2}/K_2)W^*$. As Equation 5 predicts the existence of a maximum v_{ATP} around a value of W* close to unity, the energy consumption will increase linearly from W* = 0 to its maximum value near W* = 1 and will then drop precipitously toward zero when W* tends to unity. In these conditions, the

first-order kinetics of E_2 largely governs the profile of the energy consumption at steady state (Fig. 4, *curve a*). Equations 6 and 8 further indicate that the maximum in v_{ATP} will be shifted to smaller values of the fraction of modified protein as the ratio V_{M1}/V_{M2} decreases below unity; at the same time, the rising linear part of the curve will acquire a hyperbolic character. A mirror situation holds when the inverse situation with $K_1 = 10$, $K_2 = 0.1$ is considered (Fig. 4, *curve b*).

Application to Experimental Systems

We now apply the analysis developed under "Theory" to two experimental systems for which phosphorylation-dephosphorylation has been determined in the presence of both kinase and phosphatase. In each system, we show how Equation 2 can be used to predict the amount of protein modified at steady state and determine, by means of the equations of Table I, the energy expenditure as a function of the fraction of modified protein.

Analysis of the Phosphorylation of a Synthetic Nonapeptide—The nonapeptide phosphorylation system developed by Shacter *et al.* (12, 13) represents a particularly appropriate model for monocyclic phosphorylation-dephosphorylation cascades. The interest of this *in vitro* model is the possibility to alter independently the different parameters of the modification cycle, such as the concentrations of the protein substrate and of the modification enzymes. Since cAMP activates the kinase without affecting the phosphatase activity, the system falls into the class of mechanism I. As Shacter *et al.* (13) measured energy consumption as a function of the fraction of modified substrate and obtained an empirical linear relationship, the theory described above can be applied to see whether it fits the experimental data obtained for this system.

In Fig. 5*A* are shown the experimental points obtained by Shacter *et al.* (12) for the phosphorylation of the nonapeptide as a function of cAMP which alters the activity (V_1) of the kinase. The *solid line* is obtained theoretically, by application of Equation 2, using the values determined experimentally by these authors for the various parameters (see the legend to Fig. 5); a Michaelian curve (*dotted line*) is shown for comparison.

Since the experimentally determined Hill coefficient for the activation curves is close to 1.1 (12), we represented the dependence of the kinase activity on cAMP by the Michaelian equation listed for mechanism I in Table I. The experimental value of the constant K_a for cAMP activation of the kinase depends on the total kinase concentration and varies from 0.24 to 0.46 μM as the kinase concentration is increased from

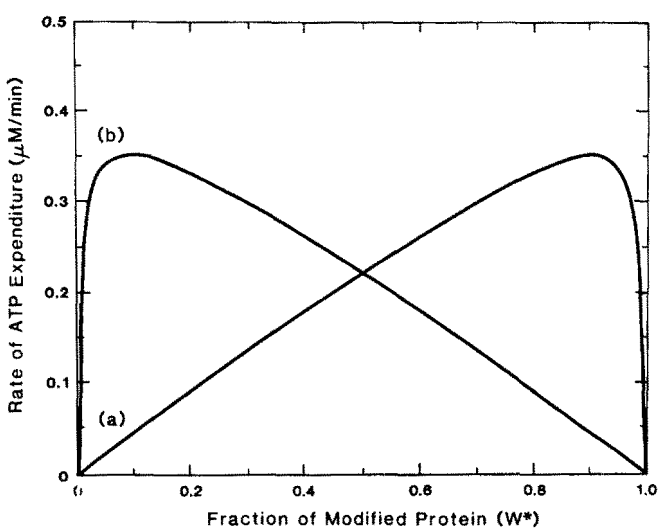

FIG. 4. **Energy expenditure at steady state as a function of fractional modification when one of the converter enzymes operates in the first-order and the other in the zero-order domain.** *Curves a* and *b* are obtained for mechanism III in Table I, according to Equation T3, for $K_1 = 0.1$, $K_2 = 10$ and $K_1 = 10$, $K_2 = 0.1$, respectively, with $V_{M1} = V_{M2} = 5$ μM/min and $K_{J1} = K_{J2} = 1$ μM.

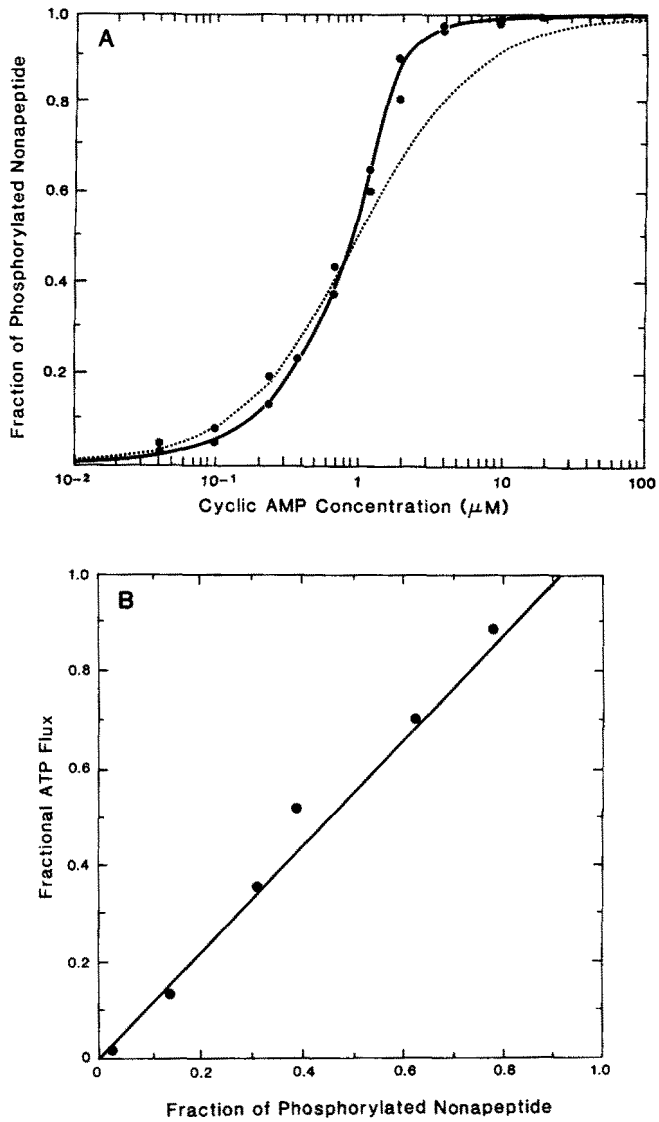

FIG. 5. **Fractional modification and energy expenditure in the synthetic nonapeptide phosphorylation system of Shacter *et al.* (12, 13).** *A*, the theoretical fraction of nonapeptide phosphorylated at steady state (*solid line*) is shown as a function of cAMP which activates the protein kinase. Redrawn from Fig. 5 of Ref. 12 for comparison are the experimental points obtained by Shacter *et al.* in duplicate experiments. The theoretical curve is generated according to Equation 2 for parameter values based on the data of Shacter *et al.*: $K_1 = 0.026$, $K_2 = 53$, $V_{M1} = 0.194$ μM/s, $V_2 = 1.5$ μM/s. The maximum rate of the protein kinase was obtained by multiplying the rate constant given by Shacter *et al.* by twice the amount of kinase used in the experiments, *i.e.* 4.4 nM. The values for K_1 and K_2 were obtained by dividing the Michaelis constants of kinase and phosphatase by the concentration of nonapeptide used in the experiments, *i.e.* 10 μM. The activation of the kinase by cAMP (J) was taken to obey the equation $V_1 = V_{M1}J/(K_{J1} + J)$ with $K_{J1} = 1.1$ μM. The *dotted line* represents a Michaelian reference curve. *B*, fractional ATP expenditure as a function of the fraction of phosphorylated nonapeptide at steady state. The theoretical curve (*solid line*) was obtained according to Equation 9, as described in the text, for the parameter values of *A*. Experimental points obtained by Shacter *et al.* were redrawn, for comparison from Fig. 3 of Ref. 13.

0.73 to 2 nM (12). The actual experiments on the nonapeptide phosphorylation were carried out at a kinase concentration of 4 nM, but no experimental value for K_a was given for these conditions. We have thus taken the value $K_a = 1.1$ μM, which yields best agreement with the experimental data on the nonapeptide phosphorylation (Fig. 5*A*). This value of K_a differs from that of 0.26 μM utilized by Shacter *et al.* (12) in their own numerical simulations. Our theoretical treatment thus predicts a higher value for the cAMP concentration giving half-maximum activation when the kinase concentration is raised to 4 nM.

Energy Expenditure as a Function of Fractional Phosphorylation—The rate of ATP expenditure in the nonapeptide phosphorylation system is given by Equation T1 of Table I. This equation reduces here to a linear form as the ratio (K_1) of the Michaelis constant of the kinase divided through the nonapeptide concentration is of the order of 0.026, whereas the corresponding constant (K_2) for the phosphatase is 60. Shacter *et al.* (13) have determined experimentally the energy consumption as a function of the fraction of nonapeptide phosphorylated at steady state. They normalized the values for the ATP consumption rate by dividing them through the rate obtained at the highest cAMP concentration tested, *i.e.* 10 μM. For this effector value, the fraction of phosphorylated nonapeptide at steady state reaches the value of 0.91. If we normalize the energy consumption rate $v_{ATP} = (V_2/K_2)W^*$ in a similar manner, we obtain the following linear relation.

$$\frac{v_{ATP}}{(v_{ATP})_{W^*=0.91}} = 1.1\ W^* \tag{9}$$

The corresponding theoretical curve (*solid line*) is shown in Fig. 5*B*, together with the experimental data (13). The predicted slope of the normalized energy expenditure curve (1.1 in this case) should decrease and approach unity as the transition moves toward total phosphorylation of the nonapeptide.

Thus, the present theoretical treatment explains the linear relationship observed in the experiments of Shacter *et al.* (13). However, the theory clearly indicates that this is not a general relationship but a special case of a particular mechanism for the control of the modification cycle.

Isocitrate Dehydrogenase Phosphorylation in E. coli—In addition to the synthetic peptide system described above, there is an *in vivo* enzyme system to which the present analysis can also be applied. This is the phosphorylation of isocitrate dehydrogenase in *E. coli.* The latter system, which occupies a central role in control of bacterial metabolism (22, 23), has previously been shown to exhibit zero-order ultrasensitivity in covalent modification (17). We shall use this system to contrast energy expenditure in zero-order as compared to first-order kinetics.

Isocitrate dehydrogenase undergoes a transition from a phosphorylated to an active, dephosphorylated state when the concentration of the effector 3-phosphoglycerate (3-PG)[1] is increased (17, 24). This effector activates the phosphatase and inhibits the kinase. The transition to the dephosphorylated form of isocitrate dehydrogenase was studied at two isocitrate dehydrogenase concentrations, 0.7 and 18 μM (17). In agreement with theoretical predictions, the transition was steeper in the latter situation ($R_J = 9$) than in the former one ($R_J = 39$) owing to the fact that the phosphatase operates in the zero-order domain at the higher concentration. (The ratio R_J of the J concentrations yielding 90 and 10% of the maximum response is 81 for a Michaelian curve; ultrasensitivity obtains when $R_J < 81$ (14–16, 25).) Part of the ultrasensitivity could also be attributed to the multistep effect (15, 25) resulting from the simultaneous action of 3-PG on both kinase and phosphatase.

In the present situation, W and W* in the scheme of Fig. 1 represent the phosphorylated and nonphosphorylated forms of isocitrate dehydrogenase, respectively, since an increase in the effector 3-PG produces a shift to the dephosphorylated state. Moreover, 3-PG acts precisely according to mechanism III since it activates E_1 (here, the phosphatase) and inhibits E_2 (the kinase).

LaPorte and Koshland (17) have obtained an equation describing the activation of the phosphatase and the inhibition of the kinase by the effector 3-PG. Although the mechanism of inhibition of the kinase remains unclear, we shall assume that 3-PG acts by decreasing the maximum rate of the enzyme without affecting the K_m. The ratio V_1/V_2 is then given by Equation 10.

$$\frac{V_1}{V_2} = \frac{V_{M1}}{V_{M2}} \cdot \frac{K_A + \beta[\text{3-PG}]}{K_A + [\text{3-PG}]} \cdot \frac{K_I + [\text{3-PG}]}{K_I} \tag{10}$$

V_{M2} is the maximum rate of the kinase in the absence of 3-PG, K_A is the activation constant of 3-PG for the phosphatase, and K_I is the inhibition constant of the effector for the kinase. As 3-PG is a nonessential activator of the phosphatase, the maximum rate of the enzyme in the absence of effector, *i.e.* V_{M1}, is multiplied by the activation factor β at saturation by 3-PG.

Equation 10 and the parameter values used in Fig. 6 originate from published experiments (17). The value of the ratio V_{M2}/K_{m2} was given there as an apparent first-order rate constant for the kinase. Here, we need to ascertain the values of both V_{M2} and K_{m2} to apply the equations which govern energy expenditure. The maximum rate of isocitrate dehydrogenase-kinase is of the order of 300 nmol/mg of enzyme/min, which corresponds to a rate of 4.5 μM/min.[2] This figure yields a value close to 13.6 μM for the K_m of the kinase and supports the assumption that the enzyme operates in the first-order domain when the total amount of isocitrate dehydrogenase is 0.7 μM; this is no longer a good approximation when the isocitrate dehydrogenase concentration is raised to 18 μM.

The theoretical curve yielding the fraction of dephosphoisocitrate dehydrogenase as a function of 3-PG can be obtained from Equation 2 in which the ratio V_1/V_2 of phosphatase to kinase rates is given by Equation 10. Together with the experimental data, this curve is shown by the *solid line* in Fig. 6*A*, for the parameter values determined experimentally, when isocitrate dehydrogenase concentration is 0.7 μM. In that case, the reduced Michaelis constants of phosphatase and kinase are $K_1 = 4.3$ and $K_2 = 19.4$, respectively. Even though the two enzymes operate in the first-order domain, due to the multistep action of 3-PG on both kinase and phosphatase, a slight ultrasensitivity is observed. The value of 31.5 for R_J compares with the value of 39 determined experimentally. Moreover, the fraction of dephosphorylated isocitrate dehydrogenase determined theoretically goes from 15% in the absence of 3-PG to 91% with 7 mM 3-PG. These values compare well with the observed values, which are close to 13 and 90%, respectively. That a significant fraction of isocitrate dehydrogenase is dephosphorylated in the absence of 3-PG is due to the basal activity of the phosphatase.

The transition curve obtained from Equations 2 and 10 for a total isocitrate dehydrogenase concentration of 18 μM compares well with the experimentally determined dephosphorylation induced by 3-PG under these conditions (data not shown). The theoretically predicted value of 6.6 for R_J is

[1] The abbreviation used is: 3-PG, 3-phosphoglycerate.

[2] D. C. LaPorte and D. E. Koshland, Jr., unpublished results.

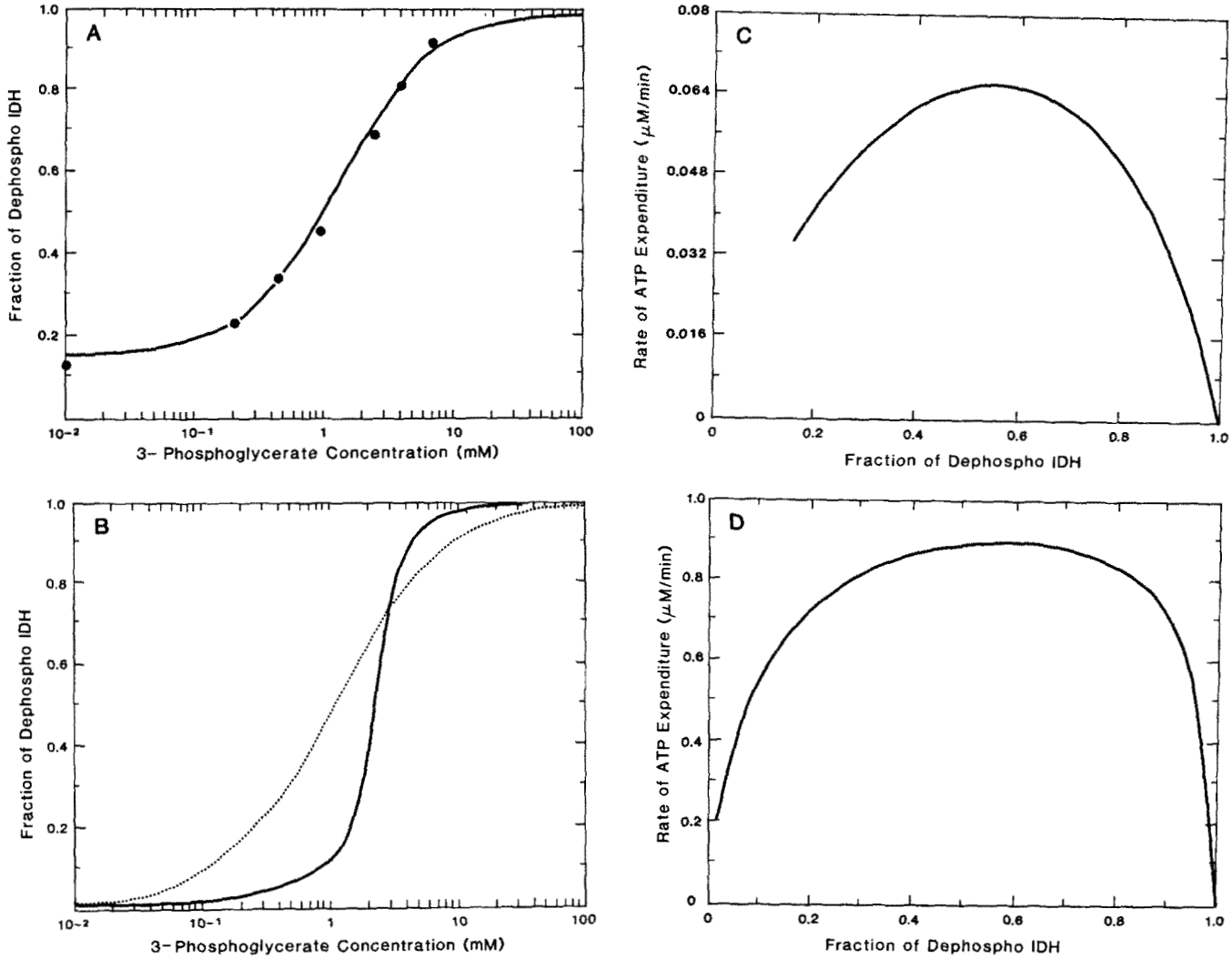

FIG. 6. **Dephosphorylation of isocitrate dehydrogenase in *E. coli*: zero-order ultrasensitivity and energy expenditure at steady state.** *A* and *B* represent the fraction of dephospho-isocitrate dehydrogenase as a function of 3-phosphoglycerate concentration, for a total isocitrate dehydrogenase concentration (*i.e.* phosphorylated plus dephosphorylated forms) of 0.7 and 36 μM, respectively; the latter value corresponds to the intracellular concentration of the enzyme. The effector 3-PG activates isocitrate dehydrogenase-phosphatase and inhibits isocitrate dehydrogenase-kinase according to Equation 10. The curves were generated according to Equations 2 and 10 for the following parameter values which are close to those determined in the experiments (17): $K_A = 3.2$ mM, $\beta = 10$, $K_I = 1.3$ mM, $K_{m1} = 3$ μM, $K_{m2} = 13.6$ μM, $V_{M1} = 0.21$ μM/min, $V_{M2} = 4.5$ μM/min (see text). For these values, the reduced Michaelis constants of phosphatase and kinase are: $K_1 = 4.3$, $K_2 = 19.43$ in *A* and $K_1 = 0.0833$, $K_2 = 0.377$ in *B*. Experimental points in *A*, redrawn from Fig. 3 of Ref. 17, were obtained by Laporte and Koshland at 0.7 μM isocitrate dehydrogenase (the point shown at 0.01 mM 3-phosphoglycerate was obtained in the absence of this effector). The *dotted line* in *B* represents the Michaelian reference curve. *C* and *D* are theoretical predictions of the energy expenditure at steady state as a function of the fraction of dephospho-isocitrate dehydrogenase at 0.7 and 36 μM isocitrate dehydrogenase, respectively. The curves in *C* and *D* are generated for the parameter values of *A* and *B*, respectively, according to Equations 11 and 13.

slightly smaller than the experimental value ($R_J = 9$). Here, the predicted transition to dephospho-isocitrate dehydrogenase goes from 3.2 to 94% when 3-PG is varied from 0 to 7 mM; these values compare with the variation from 4% to some 90% observed for the fraction of dephospho-isocitrate dehydrogenase in these conditions (17). The theoretical variation of the fraction of dephospho-isocitrate dehydrogenase in the absence of 3-PG is given in Fig. 7 as a function of the total dehydrogenase concentration.

In Fig. 6*B*, the theoretical dephosphorylation curve of isocitrate dehydrogenase is shown when the concentration is raised to 36 μM, a minimal value for the intracellular concentration of the protein. Here, the reduced Michaelis constants of phosphatase and kinase are $K_1 = 0.083$ and $K_2 = 0.377$, respectively, which indicate zero-order ultrasensitivity. The predicted value of R_J, in this case, is 4.4.

Energy Expenditure at Steady State as a Function of the Fraction of Dephospho-Isocitrate Dehydrogenase—In order to predict the energy consumption at steady state in the isocitrate dehydrogenase phosphorylation system, an equation similar to Equation T3 should be used; but we must take into account the facts that K_A does not equal K_I and that isocitrate dehydrogenase-phosphatase possesses a basal activity in the absence of 3-PG. To obtain the energy expenditure as a function of the fraction of the dephospho-isocitrate dehydrogenase, W*, we combine Equations 4 and 10 to obtain a second-degree equation which yields the effector concentration corresponding to a particular value of W* at steady state. This concentration, J_{W^*}, which yields the appropriate value of V_1/V_2, is given in Equation 11,

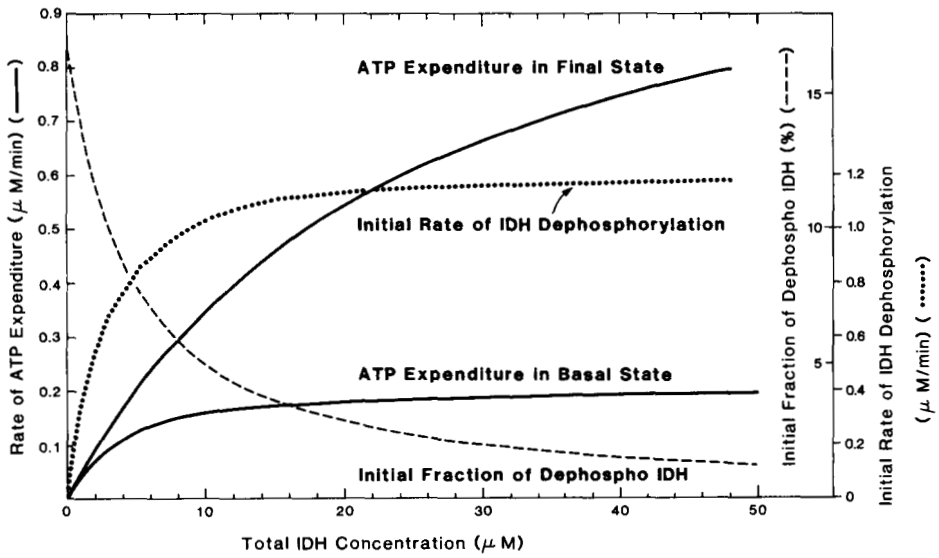

FIG. 7. **Energy expenditure and rate of evolution to steady state in the isocitrate dehydrogenase dephosphorylation system as a function of total isocitrate dehydrogenase concentration.** The initial and final values of the rate of ATP expenditure (*solid lines*) are determined by means of Equations 11–13 assuming that the system evolves from an initial state in which the concentration of 3-PG is nil to a state in which the concentration of the effector yields 90% dephospho-isocitrate dehydrogenase (*IDH*) at steady state. The initial fraction of dephospho-isocitrate dehydrogenase is indicated (*dashed line*), together with the initial rate of evolution toward the final steady state (*dotted line*), as determined from Equation 14. Parameter values are those of Fig. 6; the reduced constants K_1 and K_2 are obtained by dividing the Michaelis constants K_{m1} and K_{m2} by the total concentration of isocitrate dehydrogenase.

$$J_{W^*} = \frac{-\eta + \sqrt{\eta^2 - 4(V_{M1}/V_{M2})((V_{M1}/V_{M2}) - (V_1/V_2)K_AK_I)}}{2(V_{M1}/V_{M2})} \quad (11)$$

where $\eta = (V_{M1}/V_{M2})(\beta K_I + K_A) - (V_1/V_2)K_I$. Then, the energy expended in maintaining at steady state the fraction W* of dephospho-isocitrate dehydrogenase is given by either Equation 12 or 13.

$$v_{ATP} = V_{M1}\left(\frac{K_A + \beta J_{W^*}}{K_A + J_{W^*}}\right)\left(\frac{1 - W^*}{K_1 + 1 - W^*}\right) \quad (12)$$

$$v_{ATP} = V_{M2}\left(\frac{K_I}{K_I + J_{W^*}}\right)\left(\frac{W^*}{K_2 + W^*}\right) \quad (13)$$

The variation of energy consumption with the fraction of isocitrate dehydrogenase dephosphorylated at steady state is shown in Fig. 6 (*C* and *D*) for the concentrations of dehydrogenase considered in *A* and *B*, *i.e.* 0.7 and 36 μM, respectively. Here, as for mechanism III considered under "Energy Consumption at Steady State in the Covalent Modification Cycle," the energy consumption follows a bell-shaped curve as the amount of dephospho-isocitrate dehydrogenase increases to unity. As the maximum rate and Michaelis constant of the phosphatase differ from the corresponding constants for the kinase, the curves are no longer symmetric as in Figs. 2 and 3. As expected from the latter figures, the comparison of curves in Fig. 6 (*C* and *D*) shows that at 36 μM isocitrate dehydrogenase, the energy consumption varies in a steeper manner at both low and high values of the fraction of dephospho-isocitrate dehydrogenase; whereas at medium values of the fraction of dephosphoenzyme, the curve flattens. In addition to their distinctive shapes, the curves of Fig. 6 (*C* and *D*) also differ by the absolute amount of energy expended. It is higher at the higher isocitrate dehydrogenase concentrations, but the increased expenditure is then accompanied by an increased rate of evolution toward the steady state.

The initial rate of evolution to a steady state corresponding to 90% dephospho-isocitrate dehydrogenase is shown in Fig. 7 as a function of the total dehydrogenase concentration, ranging from 0.2 to 50 μM. We assume that no 3-PG is present initially and that at each isocitrate dehydrogenase concentration the appropriate amount of effector, $J_{0.9}$ (calculated according to Equation 11), is added so that 90% of isocitrate dehydrogenase is dephosphorylated at steady state. The initial fraction of dephospho-isocitrate dehydrogenase, W_i^*, decreases from 17% to a little more than 1% in these conditions (*dashed line* in Fig. 7). The initial rate of increase in the concentration of dephospho-isocitrate dehydrogenase is calculated according to Equation 14.

$$\frac{d[W^*]}{dt} = V_{M1}\left(\frac{K_A + \beta J_{0.9}}{K_A + J_{0.9}}\right)\left(\frac{0.1}{K_1 + 0.1}\right) - V_{M2}\left(\frac{K_I}{K_I + J_{0.9}}\right)\left(\frac{0.9}{K_2 + 0.9}\right) \quad (14)$$

Also shown in Fig. 7 are the energy expended at each total isocitrate dehydrogenase concentration for maintaining the steady-state fraction of 90% dephospho-isocitrate dehydrogenase as well as the energy expended for maintaining the initial steady state in the absence of 3-PG. The data indicate a fast rise in time responsiveness, measured by the initial rate of dephosphorylation, as the concentration of isocitrate dehydrogenase rises; that rise subsides and the rate saturates as the values of K_1 and K_2 diminish upon increasing the dehydrogenase level. Both the initial rate of isocitrate dehydrogenase dephosphorylation and the ATP expenditure at final steady state increase as the dehydrogenase level is increased above the K_m of the converter enzymes.

DISCUSSION

In biochemical systems controlled by covalent modification, the maintenance of modified protein at steady state requires

the expenditure of ATP. The present analysis shows that the variation of energy expenditure with the fraction of protein modified depends on the way the converter enzymes are controlled by the effector. Another important factor brought to light by this analysis is that the kinetic domain in which the converter enzymes operate, whether first order or zero order, has great influence on the energy expenditure profile.

The method devised is very simple and can be applied to any regulatory system based on covalent modification. It involves (*a*) determination of the level of covalent modification of the target protein by the two-step procedure summarized below and (*b*) determination of the rate of energy utilization by the equations of Table I. The two-step procedure involves (i) obtaining the dependence of the ratio of modification rates on the effector concentration; and (ii) inserting this value in Equation 2, which yields the fraction of protein modified at steady state as a function of this ratio. The combination of these two steps yields the fraction of protein modified at steady state as a function of the effector. The first step of the procedure should be omitted were the ratio of modification rates altered directly by changing the concentration of the converter enzymes, as performed recently for the demonstration of zero-order ultrasensitivity in the reversible phosphorylation of glycogen phosphorylase (18).

For simplicity in exposition, we shall summarize the steady-state results, which are general for all covalent systems, in terms of the most common type of covalent modification, *i.e.* phosphorylation. When an effector (such as cAMP) activates the kinase without affecting the phosphatase, energy consumption at steady state increases in a hyperbolic manner with the fraction of phosphorylated protein. This dependence is linear when the phosphatase operates in the first-order domain, regardless of the kinetic order of the kinase. When the effector inhibits the phosphatase without affecting the kinase, the energy expenditure decreases in a hyperbolic manner as the fraction of protein phosphorylated approaches unity. The energy dependence in this case becomes linear when the enzyme which escapes metabolic control, *i.e.* the kinase, operates in the first-order domain regardless of the kinetic order of the phosphatase.

When the effector simultaneously activates the kinase and inhibits the phosphatase, the energy consumption at steady state follows a bell-shaped curve and goes to zero at both extremes, *i.e.* when the fraction of phosphorylated protein approaches either zero or unity. Energy consumption rises sharply at these extremes and reaches an intermediary plateau when both kinase and phosphatase are in the zero-order domain; the bell shape is smoother when both enzymes operate with first-order kinetics. When the kinase is in the first-order domain and the phosphatase in the zero-order domain, the maximum of the curve is displaced toward small values of the fractional phosphorylation and the major portion of the curve consists in a linear decrease of energy consumption as the fraction of modified protein increases to unity. The reverse situation holds when the kinase is saturated by the substrate, whereas the phosphatase operates with first-order kinetics.

In all cases, the maximum energy expenditure at steady state is limited to the maximum rate of the converter enzyme with the lowest maximum activity. Thus, in the case of a very active kinase and a sluggish phosphatase, the maximum energy expenditure will eventually be dictated by the phosphatase.

Applications to Experimental Systems—The present results account for the linear relationship obtained experimentally by Shacter *et al.* (13) in the phosphorylation of a synthetic nonapeptide, but indicate theirs is a special case. In that system, the increase in steady-state phosphorylation is obtained by activation of the kinase by cAMP in the presence of a phosphatase whose activity is not affected by the regulatory ligand. At the nonapeptide concentration tested, the kinase was saturated by the substrate, whereas the phosphatase remained in the linear kinetic range. Thus, the above analysis predicts a linear increase of energy consumption at steady state with the fraction of modified protein, in good agreement with the experimental data obtained by Shacter *et al.* (see Fig. 5*B*). As made clear by our theoretical analysis, however, a linear increase of energy expenditure with fractional phosphorylation represents but one particular situation and should not be expected to hold generally.

The lack of generality of a linear relationship between energy consumption and fractional modification at steady state is further illustrated by our analysis of a second experimental system, the reversible phosphorylation of isocitrate dehydrogenase in *E. coli.* Here, an increase in the concentration of the effector 3-phosphoglycerate brings about dephosphorylation of the enzyme. As previously demonstrated (17), the effector simultaneously activates isocitrate dehydrogenase-phosphatase and inhibits isocitrate dehydrogenase-kinase. Applying our analysis to such a situation, we predict a bell-shaped dependence of ATP consumption on the steady-state fraction of dephosphorylated isocitrate dehydrogenase. The left and right portions of this bell-shaped curve should become steeper and the middle part flatter upon increasing the total concentration of isocitrate dehydrogenase from 0.7 μM to the intracellular concentration of 36 μM.

Advantages of Dual Control—Having shown that different mechanisms for the control of the converter enzymes lead to different relationships of energy consumption, we are led to the question of whether any of these regulatory modes of protein modification presents a general advantage. If this were the case, then one would expect that such mode of control of the converter enzymes would be favored and hence widely encountered in metabolic pathways controlled by covalent modification. Implicit in this conjecture is the assumption that the amount of energy expended in covalent modification represents a non-negligible fraction of the cell energy flux. (We shall return to this question at the end of the "Discussion.")

In mechanism I, in which one enzyme (the kinase) is activated by the effector and the second enzyme (the phosphatase) is unaffected, energy consumption rises in a hyperbolic or linear manner with the level of phosphorylated substrate. Such a mechanism would be favored in systems in which the protein substrate remains most of the time in the dephosphorylated state. Alternatively, in mechanism II, in which there is inhibition of the phosphatase with no effector action on the kinase, energy consumption decreases in a hyperbolic or linear manner with fractional phosphorylation. Such a mechanism would seem appropriate for systems in which the substrate protein is found most of the time in the phosphorylated form.

In mechanism III, where both kinase and phosphatase are controlled in opposite ways by the effector, the bell-shaped dependence of energy consumption on fractional modification suggests that such situations would be mostly appropriate for biochemical systems in which the protein substrate alternates during significant portions of its metabolic life between the phosphorylated and dephosphorylated states.

Such a situation of dual control by the effector appears to provide a mechanism which combines metabolic flexibility with a relative decrease in energy consumption. Only during the transition from a low to a high level of protein phospho-

rylation would such systems pass through a region of high ATP expenditure; being only transient, such rise in energy consumption would represent a small price to pay when compared to the lowered rate of ATP expenditure at steady state.

In addition to the possible energetic saving, dual regulation of the two converter enzymes by a single effector provides a number of physiologically significant advantages: (i) a smaller change in stimulus is required to bring about the transition to a given level of protein modification; and (ii) simultaneous activation of the forward reaction and inhibition of the reverse reaction accelerate the evolution toward the steady state and thus increase the time responsiveness of the modification system. In view of these cumulated properties, dual regulation of the converter enzymes by a single effector appears to be a most efficient mechanism for the control of covalent modification systems. Dual control is encountered in a number of biochemical systems such as the phosphorylation of isocitrate dehydrogenase in *E. coli* (see "Isocitrate Dehydrogenase Phosphorylation in *E. coli*"), where 3-PG activates the phosphatase and inhibits the kinase. In glycogen metabolism, cAMP activates protein kinase, but this action also results in the activation through phosphorylation of a protein inhibitor of protein phosphatase (19–21). Both the kinase and the phosphatase are therefore under control of cAMP. In bacterial chemotaxis, reversible methylation of the chemoreceptors plays a primary role in sensory adaptation. Here, an attractant stimulus activates the methyltransferase and inhibits the methylesterase (26, 27). Dual control of the two converter enzymes by a single effector likely occurs in other metabolic systems regulated by covalent modification.

Relationship of Ultrasensitivity to Energy Consumption—The theoretical relationships derived in this paper permit one to determine the link between zero-order ultrasensitivity in covalent modification and energy consumption. When the total concentration of the protein substrate is increased with respect to that of the converter enzymes, the latter become saturated and the transition curve becomes steeper and steeper. This zero-order effect provides a "covalent switch mechanism" (see *curves a* and *b* in Fig. 1) which may underlie abrupt transitions in a large number of normal and pathological cellular processes (14–16). The increase in sensitivity implied by this phenomenon is accompanied by an increase in energy consumption (compare *curves III* in Figs. 2 and 3, as well as Fig. 6, *C* and *D*).

Increased energy expenditure can be viewed as the price that must be paid for both the gain in sensitivity resulting from the zero-order effect and the increased rate of attainment of the steady state (Fig. 7). Enhanced time responsiveness and ultrasensitivity could combine with a relative reduction in energy expenditure were the modification system operating according to the mechanism of dual control of the converter enzymes by a single effector. Indeed, ATP expenditure can be significantly decreased if the system then operates either at very low or very high values of fractional modification at steady state.

A further advantage of zero-order untrasensitivity should be stressed in light of this discussion. As illustrated in Fig. 1, at a given value of the ratio of modification rates V_1/V_2 distant from the mid-transition value where equal amounts of modified and unmodified protein coexist, zero-order ultrasensitivity allows the transition to either one of these two states to be brought toward completion. Thus, some 15% of isocitrate dehydrogenase exists in the dephosphorylated state in the absence of 3-phosphoglycerate in *E. coli* when the isocitrate dehydrogenase concentration is 0.7 μM; this fraction drops to 4% when the dehydrogenase concentration is raised to 18 μM (see also Fig. 7). In addition to lowering the relative energy expenditure, zero-order ultrasensitivity enhances the all-or-none nature of the switch inherent in covalent modification.

Energy Consumption in Animal Cells—In discussing the energy consumption associated with covalent modification cycles, the question arises as to what fraction of the total ATP flux is channeled into such metabolic processes. In order to estimate this, we must have a reasonable value for total ATP consumption. This value is in some dispute, and part of the problem is created by different units and standards. Most data are given in the literature as units of enzyme activity/unit of tissue or cells, wet or dry weight. In expressing these figures in molar/minutes so as to compare the rates of covalent modification and ATP regeneration, we had to transform weights into volumes. This is usually done by taking 1 g of wet weight as corresponding to 0.5 or 1 ml of intracellular water. Given these rough estimates of conversion factors, errors associated with pool sizes, etc., the rates must be taken as first approximations.

That the rate of ATP production in liver and muscle is in the millimolar/minute range is indicated by calculations based on respiration measurements in various tissues or in whole animals (28). Thus, the oxygen consumption in a large number of rat tissues, determined in similar conditions, varies from 6.5–11.6 (liver) to 3.8–10.4 (heart) and 2.3–3.1 (skeletal muscle) mm^3/mg of tissue, dry weight/h. In man, the corresponding figures are 6.3 (liver) and 10.4 (heart). Assuming that 1 mg of dry tissue corresponds to 3 mg of intracellular water and that 6 mol of ATP are produced per mol of O_2, the above figures yield a value of 15 mM/min for the ATP production rate associated with an oxygen consumption of 10 mm^3/mg of tissue, dry weight/h in liver. In skeletal muscle at rest, the quantity of oxygen consumed is smaller by a factor of 3–5 (see above). This would yield an ATP production rate of 3–5 mM/min in muscle. Such a figure holds with that of 2 mM/min obtained by others (18) on the basis of similar calculations.

Another basis for estimating global ATP production rates in human tissues is provided by the well-known data (29) on ATP utilization in man. Thus, Erecinska and Wilson (30) indicate that a man of 68 kg uses 40 kg of ATP in 24 h, *i.e.* 0.03 kg of ATP/min at rest and up to 0.6 kg of ATP/min when exercising. These data can be arrived at on the basis of oxygen consumption measurements of 220 and 4000 mm^3/g, wet weight/h for a resting or exercising man, respectively (28). When transformed into ATP production rates, the above measurements yield the values of 1.2 mM/min at rest and 24 mM/min during exercise.

Finally, as to isolated cells, the rate of ATP production has been determined in human platelets (31, 32). These cells consume ATP at a basal rate of 4–6 μmol of ATP/min/10^{11} platelets. Assuming that 7.8×10^{10} platelets correspond to 1-ml volume of cells (33), these figures yield a basal ATP turnover rate close to 4 mM/min. On the basis of cell volume, Verhoeven *et al.* (32) indicate that this number is close to the values found in human neutrophils and lymphocytes, but larger by 2 orders of magnitude than in rabbit and human erythrocytes.

Conflicting with these numbers, Shacter and co-workers (13, 34) estimate ATP expenditure as 2 M/min in liver tissue. In view of the above results and as also pointed out by Meinke *et al.* (18), this value appears to be too high by 2–3 orders of magnitude. It is based on figures cited by Lipmann (35), derived from experiments carried out by means of ^{31}P NMR

spectroscopy in perfused mouse liver (36). Rather than the value of 1 mmol/g, wet weight/min given in Ref. 35 and yielding the value of 2 M/min, the original data of McLaughlin *et al.* (36) appear to indicate that the ATP regeneration rate in this system is 0.75 mM/min. Therefore, the conclusion (13, 34) that the phosphorylation reactions involved in the control of pyruvate kinase and glycogen phosphorylase utilize only 0.02% of ATP turnover, based on the 2 M/min estimate, must be revised upwards by 2–3 orders of magnitude.

Estimation of ATP Turnover Rates in Microorganisms—Chapman and Atkinson (37) have given a detailed review of ATP turnover rates in microorganisms. The data they collected generally indicate a linear relationship between ATP regeneration and growth rate. In *E. coli*, at three different growth rates, the value reported for ATP production was close to 2000 μmol/min × g, dry weight. Assuming that 1 g, dry weight, correspond to 3 g of intracellular water, the latter value yields a rate of ATP regeneration of 0.8 M/min. This value is in good agreement with those of 0.9 and 1.1 M/min reported by Walsh and Koshland (22) on the basis of two independent series of measurements in *E. coli*.

The maximum ATP production rate in bacteria is therefore larger than that in animal tissues. Data collected for various microorganisms (37) nevertheless indicate that ATP turnover rates may vary by a factor of up to 50 in the range 20 mM to 1 M/min. The lower value corresponds to the maximum ATP turnover observed in animal tissues such as liver or exercising muscle.

ATP Utilization in Covalent Modification—In order to compare energy utilization in covalent modification with ATP turnover, we need to estimate rates in some known modification systems. There are many known phosphorylation reactions catalyzed by different protein kinases. One of the most active is phosphorylase kinase. This enzyme is present in rabbit muscle at an intracellular concentration of 0.8 mg/ml (20). Given a maximum rate close to 12.5 μmol/min × mg of enzyme at pH 8.2 (38, 39), the maximum rate of phosphorylation of phosphorylase *b* should be close to 10 mM/min; a similar value obtains for the phosphorylated (*i.e.* activated) enzyme at physiological pH 6.8 (38). This value is slightly larger than those mentioned previously (13, 18).

The catalytic subunit of the cAMP-dependent protein kinase has a turnover number close to that of phosphorylase kinase (39), but a concentration smaller by a factor of 10, *i.e.* close to 0.4 μM (20). The maximum rate of phosphorylation by this enzyme therefore should be of the order of 1 mM/min. Hofmann *et al.* (40) determined the activity of this kinase in skeletal muscle, heart, kidney, liver, and brain and found it to be about 150 μM/min × kg of these tissues, wet weight, *i.e.* of the order of 0.2 mM/min. The activity of protein kinase C was found to be larger by 1 order of magnitude than that of the cAMP-dependent kinase in cells such as human platelets (41).

For the protein phosphatase 1 active in glycogen metabolism, dephosphorylation rates (in micromolar/minutes) range from 16 in rabbit or rat liver to 33 in rabbit skeletal muscle (42). Another set of data (21) yields a rate of 50 μM/min. Higher rates are obtained on the basis of purified enzyme activities. Thus, the data of Resink *et al.* (43) yield dephosphorylation rates ranging from 0.35 to 0.85 mM/min.

A different instance in which covalent modification rates can be estimated *in vivo* is that of the reversible methylation of chemoreceptors in bacterial sensing. Fifty percent of the methyl groups of the bacterial receptors turn over in 15 min (27). There are 3500 receptors/cell (44) and an average of 1.5 methyl groups/receptor in the absence of chemotactic stimulation. These data yield a turnover number close to three methyl groups/cell × s, corresponding to a basal methylation rate of 0.2 μM/min when the volume of the bacterial cell is taken as 1.5×10^{-15} liter. Given that 6 mol of ATP are used in the synthesis and regeneration of 1 mol of *S*-adenosylmethionine, this rate corresponds to an ATP utilization of 1.2 μM/min. During chemotactic stimulation, this rate can go up transiently by a factor of 5.

Comparison of Covalent Modification Rates with ATP Turnover—In view of the above figures, we are led to the conclusion that large phosphorylation rates such as that of 1–10 mM/min reported for phosphorylase kinase represent a sizable portion of ATP turnover in muscle. Even though other modification reactions may operate at lower pace, the rates of ATP expenditure in covalent modification are by no means negligible with respect to the total ATP flux in animal tissues. In microorganisms, they represent a smaller part of the total ATP flux; but such cells have multiple needs such as motility, transport, sensing, etc., not all of which are needed in a specialized cell of a multicellular organism. Therefore, the enhanced turnover of ATP in bacteria should not be taken as indicative of energetic surplus.

Examples of detailed comparison of ATP consumption in various cellular processes, including protein covalent modification, are scarce. One exception is provided by studies on energy consumption in resting and stimulated blood platelets. Upon stimulation by thrombin, platelets undergo shape change, aggregation, protein phosphorylation, and secretion. The increase in ATP consumption over the basal rate of 4 mM/min (31, 32) was found to be of the order of 6 mM ATP during the first 20 s following stimulation (45). Of these, a small but non-negligible amount of ATP was consumed in the phosphorylation of two proteins; phosphorylation of one of these proteins appears to be catalyzed by protein kinase C (41).

In covalent modification systems, energy is also being consumed by the synthesis of intracellular effectors such as cAMP and phosphatidylinositol. In the above-discussed platelet system, the turnover of cAMP has been estimated to be as high as 68 and 300 μM/min in the basal and prostacyclin-stimulated states, respectively (33). As to the phosphatidylinositol cascade which controls the activation of protein kinase C (41), measurements in platelets indicate that the rate of phosphatidylinositol depletion in the first 10 s after stimulation by thrombin is of the order of 4 mM/min (46). Although these are transient effects, when considering energy expenditure the cost of generating appropriate effector levels is not necessarily negligible.

Not only is the cost in energy of certain vital enzymes appreciable, as discussed; but another factor must be considered, namely, the large number of reactions involving covalent modification. The finding that approximately one out of six proteins in mammalian cells is reversibly phosphorylated and that 1 out of 300 may be reversibly methylated (8) indicates the extensive prevalence of these reactions. Since the above calculation was based on one phosphate group/protein, multiple phosphorylation would mean fewer proteins, and partial phosphorylation would mean more proteins are modified. If a normal cell contains several thousand proteins, turnover rates for individual enzymes appreciably less than those cited above would still have a dramatic cumulative effect. The conclusion that any one covalent modification uses only a small fraction of the total ATP consumption rate is therefore deceptive since there are so many of these reactions.

The present analysis has brought to light the conditions that favor maximum responsiveness at minimal energy cost.

These include operation at the extremes of the modification transition rather than at intermediate levels of protein modification, as well as dual control of the modifying and demodifying enzymes by a single effector. A further role of such dual control may be to lower the steady-state rate of covalent modification and hence the rate of ATP expenditure needed for maintenance of a given amount of modified protein. Moreover, the comparison of individual covalent modification energetics with the overall ATP turnover indicates that energy conservation may be needed. In such a case, the selection of an appropriate mode of control for each covalent modification system should prove essential for optimizing energy expenditure, in parallel with the responsiveness to external stimuli and the degree of completion required for protein modification.

Acknowledgment—We wish to thank Dr. D. LaPorte for his comments on the manuscript.

REFERENCES

1. Krebs, E. G., and Beavo, J. A. (1979) *Annu. Rev. Biochem.* **48,** 923–959
2. Chock, P. B., Rhee, S. G., and Stadtman, E. R. (1980) *Annu. Rev. Biochem.* **49,** 813–843
3. Rosen, O. M., and Krebs, E. G. (eds) (1981) *Protein Phosphorylation*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
4. Cohen, P. (1982) *Nature* **296,** 613–620
5. Nestler, E. J., and Greengard, P. (1984) *Protein Phosphorylation in the Nervous System*, Wiley-Interscience, New York
6. Cohen, P. (ed) (1984) *Enzyme Regulation by Reversible Phosphorylation: Further Advances*, Elsevier Scientific Publishing Co., Amsterdam
7. Forsberg, H., Zetterqvist, Ö., and Engström, L. (1969) *Biochim. Biophys. Acta* **181,** 171–175
8. Chelsky, D., Ruskin, B., and Koshland, D. E., Jr. (1985) *Biochemistry* **24,** 6651–6658
9. Macfarlane, R. G. (1964) *Nature* **202,** 498–499
10. Jackson, C. M., and Nemerson, Y. (1980) *Annu. Rev. Biochem.* **49,** 765–811
11. Newsholme, E. A., and Crabtree, B. (1976) *Biochem. Soc. Symp.* **41,** 61–109
12. Shacter, E., Chock, P. B., and Stadtman, E. R. (1984) *J. Biol. Chem.* **259,** 12252–12259
13. Shacter, E., Chock, P. B., and Stadtman, E. R. (1984) *J. Biol. Chem.* **259,** 12260–12264; Correction (1985) *J. Biol. Chem.* **260,** 6501
14. Goldbeter, A., and Koshland, D. E., Jr. (1981) *Proc. Natl. Acad. Sci. U. S. A.* **78,** 6840–6844
15. Koshland, D. E., Jr., Goldbeter, A., and Stock, J. B. (1982) *Science* **217,** 220–225
16. Goldbeter, A., and Koshland, D. E., Jr. (1982) *Q. Rev. Biophys.* **15,** 555–591
17. LaPorte, D. C., and Koshland, D. E., Jr. (1983) *Nature* **305,** 286–290
18. Meinke, M. H., Bishop, J. S., and Edstrom, R. D. (1986) *Proc. Natl. Acad. Sci. U. S. A.* **83,** 2865–2868
19. Huang, F. L., and Glinsmann, W. H. (1976) *Eur. J. Biochem.* **70,** 419–426
20. Cohen, P. (1978) *Curr. Top. Cell. Regul.* **14,** 117–196
21. Lee, E. Y. C., Silberman, S. R., Ganapathi, M. K., Petrovic, S., and Paris, H. (1980) *Adv. Cyclic Nucleotide Res.* **13,** 95–131
22. Walsh, K., and Koshland, D. E., Jr. (1985) *J. Biol. Chem.* **260,** 8430–8437
23. Nimmo, H. G. (1984) in *Enzyme Regulation by Reversible Phosphorylation: Further Advances* (Cohen, P., ed) pp. 123–141, Elsevier Scientific Publishing Co., Amsterdam
24. LaPorte, D. C., and Koshland, D. E., Jr. (1982) *Nature* **300,** 458–460
25. Goldbeter, A., and Koshland, D. E., Jr. (1984) *J. Biol. Chem.* **259,** 14441–14447; Correction (1986) *J. Biol. Chem.* **261,** 4358
26. Toews, M. S., Goy, M. F., Springer, M. S., and Adler, J. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 5544–5548
27. Terwilliger, T., Wang, J. W., and Koshland, D. E., Jr. (1986) *J. Biol. Chem.* **261,** 10814–10820
28. Altman, P. L., and Dittmer, D. S. (eds) (1968) *Biological Handbooks: Metabolism*, pp. 362 and 385, Federation of American Societies for Experimental Biology, Bethesda, MD
29. Stryer, L. (1981) *Biochemistry*, Freeman, San Francisco
30. Erecinska, M., and Wilson, D. F. (1978) *Trends Biochem. Sci.* **3,** 219–223
31. Akkerman, J. W. N., Gorter, G., Schrama, L., and Holmsen, H. (1983) *Biochem. J.* **210,** 145–155
32. Verhoeven, A. J. M., Mommersteeg, M. E., and Akkerman, J.-W. N. (1984) *Biochim. Biophys. Acta* **800,** 242–250
33. Goldberg, N. D., Walseth, T. F., Eide, S. J., Krick, T. P., Kuehn, B. L., and Gander, J. E. (1984) *Adv. Cyclic Nucleotide Protein Phosphoryl. Res.* **16,** 363–379
34. Chock, P. B., Shacter, E., Jurgensen, S. R., and Rhee, S. G. (1985) *Curr. Top. Cell. Regul.* **27,** 3–12
35. Lipmann, F. (1981) *Curr. Top. Cell. Regul.* **18,** 301–311
36. McLaughlin, A. C., Takeda, H., and Chance, B. (1979) *Proc. Natl. Acad. Sci. U. S. A.* **76,** 5445–5449
37. Chapman, A. G., and Atkinson, D. E. (1977) *Adv. Microbiol. Physiol.* **15,** 253–306
38. Kemp, B. E., and John, M. J. (1981) in *Protein Phosphorylation* (Rosen, O. M., and Krebs, E. G., eds) pp. 331–342, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
39. Graves, D. J. (1983) *Methods Enzymol.* **99,** 268–278
40. Hofmann, F., Bechtel, P. J., and Krebs, E. G. (1977) *J. Biol. Chem.* **252,** 1441–1447
41. Takai, Y., Kikkawa, U., Kaibushi, K., and Nishizuka, Y. (1984) *Adv. Cyclic Nucleotide Protein Phosphoryl. Res.* **18,** 119–158
42. Cohen, P. (1983) *Methods Enzymol.* **99,** 243–250
43. Resink, T. R., Hemmings, B. A., Tung, H. Y. L., and Cohen, P. (1983) *Eur. J. Biochem.* **133,** 455–461
44. Clarke, S., and Koshland, D. E., Jr. (1979) *J. Biol. Chem.* **254,** 9695–9702
45. Verhoeven, A. J. M., Gorter, G., Mommersteeg, M. E., and Akkerman, J. W. N. (1985) *Biochem. J.* **228,** 451–462
46. Bell, R., and Majerus, P. W. (1980) *J. Biol. Chem.* **255,** 1790–1792