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Allosteric Proteins and Cellular Control Systems

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The biological activity of many proteins is controlled by specific metabolites which do not interact directly with the substrates or products of the reactions. The effect of these regulatory agents appears to result exclusively from a conformational alteration (allosteric transition) induced in the protein when it binds the agent. It is suggested that this mechanism plays an essential role in the regulation of metabolic activity and also possibly in the specific control of protein synthesis.

1. Introduction

Considerable progress has been made during the past few years in the study of regulation and control of cellular metabolism. It is now established that even in the simplest organisms, such as bacteria, complex circuits of regulation play an essential role, governing not only the rate of flow of metabolites through different pathways but also the synthesis of proteins and other macromolecules. Most of these control systems involve a sequence of reactions and interactions and their physiological diversity is extreme. However, in several instances the components of such systems have been resolved, allowing identification and study of the elementary controlling interaction. In virtually all of the systems which have been analysed in sufficient detail, this elementary interaction involves a protein endowed with a specific biological activity and an active agent, generally a low-molecular weight metabolite, in whose presence the specific process governed by this protein is either accelerated or inhibited.

It would appear, in other words, that certain proteins, acting at critical metabolic steps, are electively endowed with specific functions of regulation and coordination; through the agency of these proteins, a given biochemical reaction is eventually controlled by a metabolite acting apparently as a physiological "signal" rather than as a chemically necessary component of the reaction itself (Monod & Jacob, 1961; Jacob & Monod, 1962).

It is hardly necessary to point out the critical role, indeed the physiological necessity, of such metabolic interconnections. In this paper we will not be concerned with the physiological interpretation of individual systems but rather with the mechanism of the controlling interaction. Our aim will be to enquire whether, in spite of the extreme diversity of these systems, it may be possible to formulate certain generalizations concerning the functional structures responsible for the regulatory competence of the controlling proteins, allowing them to act as specific mediators of these essential interactions. At the outset we should like to make it clear that we will not be proposing a new theory, nor any original interpretation of individual facts, but only comparing

various examples and attempting to see to what extent and in what way a general description of these systems might be valid and useful.

For the sake of clarifying the discussion and defining the terminology to be used, it is convenient to state *a priori* some of the conclusions at which we shall arrive. This may be done in the form of a general model schematizing the functional structures of controlling proteins. These proteins are assumed to possess two, or at least two, stereospecifically different, non-overlapping receptor sites. One of these, the *active site*, binds the substrate† and is responsible for the biological activity of the protein. The other, or *allosteric site*, is complementary to the structure of another metabolite, the *allosteric effector*, which it binds specifically and reversibly. The formation of the enzyme–allosteric effector complex does not activate a reaction involving the effector itself: it is assumed only to bring about a discrete reversible alteration of the molecular structure of the protein or *allosteric transition*, which modifies the properties of the active site, changing one or several of the kinetic parameters which characterize the biological activity of the protein.

An absolutely essential, albeit negative, assumption implicit in this description is that an allosteric effector, since it binds at a site altogether distinct from the active site and since it does not participate at any stage in the reaction activated by the protein, need not bear any particular chemical or metabolic relation of any sort with the substrate itself. The specificity of any allosteric effect and its actual manifestation is therefore considered to result exclusively from the specific construction of the protein molecule itself, allowing it to undergo a particular, discrete, reversible conformational alteration, triggered by the binding of the allosteric effector. The *absence* of any inherent obligatory chemical analogy or reactivity between substrate and allosteric effector appears to be a fact of extreme biological importance, and in a sense it is the main subject of the present paper. In addition, it is evidently essential to a proper definition of allosteric effects as distinct from the action of coenzymes, secondary substrates or substrate analogues, all of which react with the substrate or substitute for the substrate and therefore must bear some structural relation with or chemical reactivity towards it. This being said, one should certainly not exclude the possibility that the action of certain coenzymes or other enzyme effectors may involve allosteric effects in addition to their classical role as transient reactants or transporters. Nor should one forget the possibility, suggested by Koshland (1958), that the binding of substrate involves an induced alteration of the shape of the enzyme site. These possibilities will be discussed only briefly in this paper which will deal exclusively with typical cases of regulatory allosteric effects, limitatively defined as above.

Since our purpose is to enquire whether any generalizations can be made concerning the functional structures of regulatory proteins, we will have to compare different systems, controlling different reactions and endowed with different physiological functions, in widely different organisms. Unfortunately, the nature of the information concerning different systems is very heterogeneous, rarely allowing detailed parallel comparisons, and the generalized picture will have to be sorted out of this experimental puzzle. We will first discuss the properties of certain bacterial enzymes that act as regulators of biosynthetic pathways. The kinetic properties of some of these systems have been well studied and their regulatory role is perfectly clear, but little is known

† In the present context, we shall use the word "substrate" in a somewhat wider sense than is usual, to designate the specific compound upon which a protein exerts its biological activity, whether or not the protein in question is an enzyme *sensu stricto* or not.

about the molecular properties of the proteins. In subsequent sections we will consider certain mammalian enzymes, subject to different regulatory effects, the precise physiological role of which is not always clear but in which conformational alterations have been directly observed. In the last section we shall discuss the validity, qualifications and limitations of the "allosteric" model, and the biological significance of this mechanism.

2. Allosteric Proteins as Metabolic Regulators

(a) *Specificity and kinetics of allosteric effects*

The biosynthetic pathways of bacteria have afforded some of the clearest instances of metabolic regulation. We refer to the so-called "feed-back" or "end-product" inhibition effect discovered by Novick & Szilard (1954), whose early observations on the synthesis of tryptophan, followed by the enzymological work of Umbarger (1956), Yates & Pardee (1956) and others, have now been extended to most, if not all, pathways leading to the synthesis of essential metabolites. Actually it appears to be a rule in bacteria that the terminal metabolite synthesized in any given pathway is a powerful and specific inhibitor of its own synthesis. It is also a rule that only one enzyme (usually the first one in each specialized pathway) is responsible for this effect.

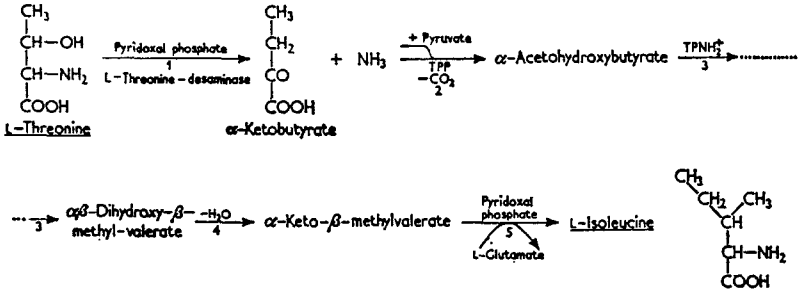

FIG. 1(a). (See p. 309.)

Several of these enzymes have now been studied in some detail and proved to possess certain remarkable and even, at first sight, paradoxical properties which as we shall see actually depend upon and reveal the allosteric construction of these proteins. Similar feed-back effects have been observed in various metabolic pathways of higher organisms.†

Six of these enzymes: threonine-deaminase (Umbarger & Brown, 1958; Changeux, 1961, 1962); aspartic-transcarbamylase (ATCase)‡ (Gerhart & Pardee, 1962); phosphoribosyl-ATP-pyrophosphorylase (PRPP-ATP-PPase) (Martin, 1962); aspartokinases I and II (Stadtman, Cohen, Le Bras & de Robichon-Szulmajster, 1961) and homoserine-dehydrogenase (Patte, Le Bras, Loviny & Cohen, 1962) will especially be mentioned here. The biosynthetic pathways in which they respectively operate are shown in Fig. 1.

† Actually, one of the very first clearly recognized instances of a regulatory feed-back mechanism of this type appears to have been the inhibition of glucose phosphorylation by phosphoglyceric acid in erythrocytes. This was described by Dische over 20 years ago (Dische, 1941) in a paper which came only very recently to our attention.

‡ The following abbreviations are used in this paper: ATCase = aspartic transcarbamylase; PRPP-ATP-PPase = phosphoribosyl-ATP-pyrophosphorylase; GDH = glutamic dehydrogenase; S-RNA = soluble RNA.

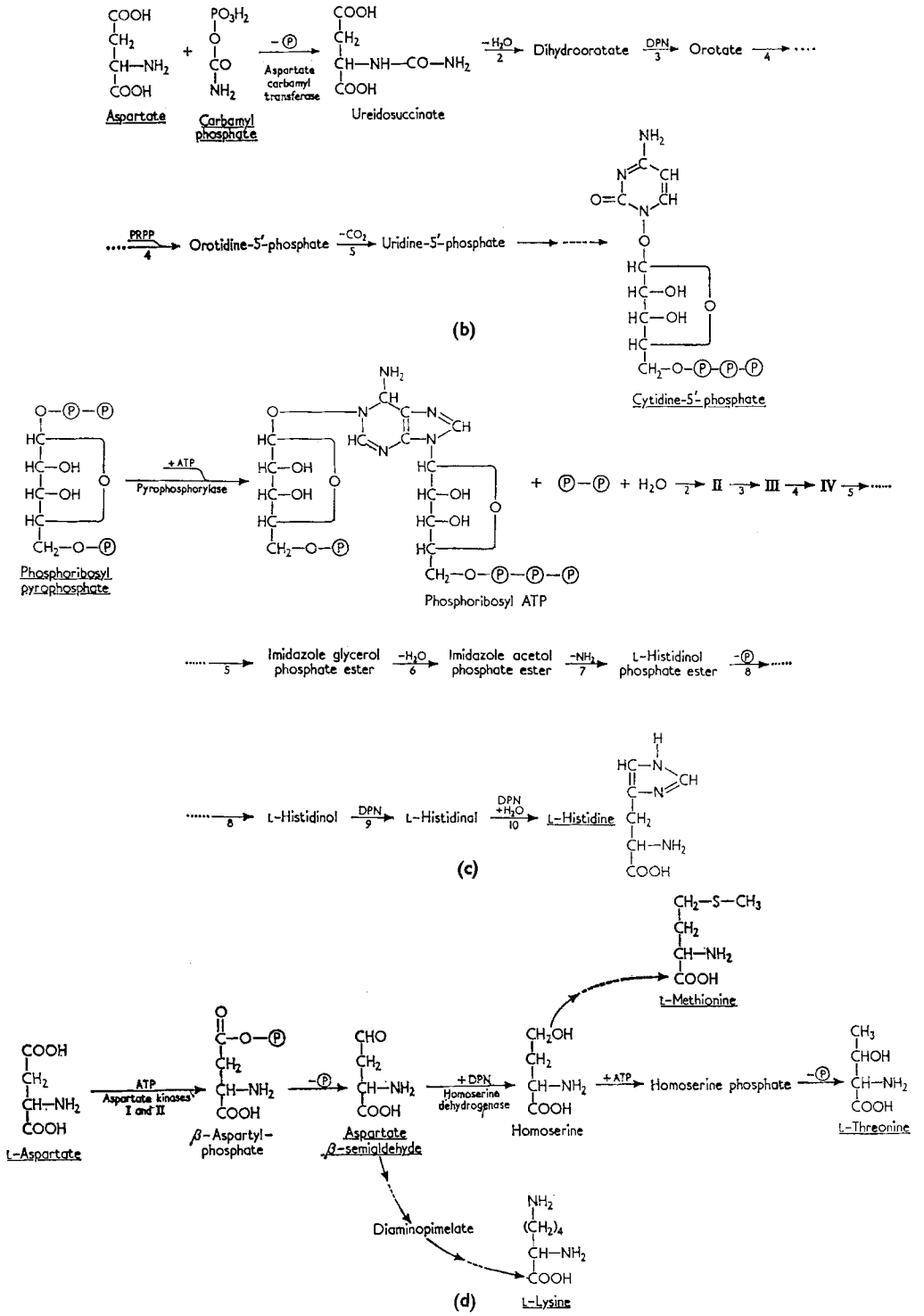

FIG. 1. (a), (b), (c), (d). Examples of biosynthetic pathways subject to the "end-product inhibition" effect. The inhibitory metabolites and the substrates of the sensitive enzyme(s) in each pathway are underlined. In the pathway beginning with aspartate, the aspartate-kinase reaction is inhibited by both L-threonine and L-lysine; the homoserine-dehydrogenase reaction is inhibited by L-threonine.

All these systems obey the rules stated above, namely:

(a) the regulatory enzymes (each of them acting immediately *after* a metabolic branching point) are all strongly and specifically inhibited by the terminal metabolite of the pathway in which each of them operates; intermediary metabolites in each pathway do not inhibit the regulatory enzyme;

(b) the enzymes which intervene *after* the regulatory one in each pathway are not significantly sensitive to inhibition by the terminal metabolite.

These facts alone suffice to demonstrate that the inhibitory effects must be due to highly specialized molecular structures present in the sensitive enzymes and cannot be accounted for by considerations of steric analogy between substrate and inhibitor. In the case of threonine-deaminase, for instance, it might be considered that substrate and inhibitor are steric analogues to the extent that both are α-amino acids; but certain α-amino acids are devoid of any inhibitory action, while others are activators, as we shall see later. Moreover, *E. coli* is known to synthesize two different threonine-deaminases, one of which, as shown by Umbarger, is a degradative enzyme and is completely insensitive to inhibition by isoleucine (Umbarger & Brown, 1957). Finally, the coexistence in *E. coli* of two different aspartokinases catalysing identical reactions, respectively inhibited by threonine and by lysine (Stadtman *et al.*, 1961), offers a striking illustration of the fact that the nature and structure of the inhibitor is, in a sense, irrelevant to the interpretation of the effect. Clearly, such an interpretation must be sought exclusively in the functional structure of the regulatory protein itself.

Since the inhibitory metabolites must act by forming a stereospecific complex with the enzyme, the first questions to consider concern the relationship of the inhibitor binding site to the substrate binding site. Clearly, the same system of binding groups† cannot be involved for both, since inhibitor and substrate are not *isosteric*, but rather *allosteric* with respect to each other. This conclusion, based exclusively upon structural considerations, has been directly confirmed by the discovery (Changeux, 1961; Gerhart & Pardee, 1961, 1962) that under certain conditions or after treatment by certain agents, or also by mutation, a regulatory enzyme may lose its sensitivity to the inhibiting metabolite while retaining its activity towards substrate. This observation has now been made with at least five different systems and therefore appears to be of great significance for the interpretation of allosteric interactions. We shall discuss it in more detail later. For the time being we use it only as proof that the binding of substrate and inhibitor do not involve the same groups.

This being established, it will be useful to distinguish *a priori* three possible types of interaction between substrate and inhibitor. These are shown schematically in Fig. 2.

In the first type, the binding sites actually overlap (although the binding *groups* are not the same). The binding of substrate and inhibitor are therefore exclusive of one another, because of *steric hindrance*. In the second type the two sites lie so close to one another that *direct interactions* (either attractive or repulsive) between substrate and inhibitor occur. In the third type no direct interactions are involved, the two sites are completely separate; the effect is therefore mediated entirely by the protein, presumably through a conformational alteration resulting from the binding of the

† We define "binding site" as the particular area covered by a substrate or effector on the protein surface; "binding groups" as the atoms or groups of the protein which form actual bonds with the substrate and/or effector.

inhibitor, i.e. through an *allosteric transition*. Let us briefly consider the predictions of each of these models as far as the kinetics of inhibition are concerned.

In the case of model I the interaction can *only* be of the "strict competitive" type; that is:

(a) the presence of inhibitor affects *exclusively* the apparent enzyme–substrate dissociation;

(b) the inhibition curve will be asymptotic to 100% at high inhibitor concentration. Therefore, any other result, such as non-competitive inhibition (apparent dissociation constant unaffected), mixed inhibition (apparent dissociation and maximal activity both altered) or incomplete competitive inhibition (inhibition curve asymptotic to a finite value), would eliminate model I.

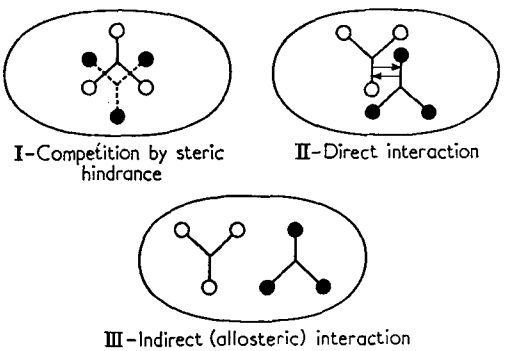

FIG. 2. Three models of interaction between a substrate and an inhibitor binding respectively with different groups on enzyme surface.

Assuming that model I did not apply, one would have to distinguish between models II and III, which is evidently far more difficult, both models being compatible with a variety of kinetics.† However, model II is more restrictive than model III since the former requires that any interaction be *reciprocal* while the latter does not. It follows from this that strictly non-competitive effects are not expected on the basis of model II, which implies that the affinities of the inhibitor for the free enzyme and for the enzyme–substrate complex should be different.

The available data concerning the kinetics of inhibition of bacterial enzymes do not in every case allow application of these criteria. However, as we shall see, these data when taken in conjunction with other lines of evidence appear to be incompatible with model I and difficult to reconcile with model II.

To begin with, we may note that in the cases of PRPP-ATP-PPase, aspartokinase II and homoserine-dehydrogenase inhibition is strictly non-competitive, eliminating model I and also contradicting model II. With aspartic-transcarbamylase and aspartokinase II, the inhibition is competitive (in the sense that only the apparent affinity is affected) but it is incomplete. This may be taken also to eliminate model I (but not model II). This evidence is somewhat questionable, however, because it has repeatedly been observed that even mild treatments (such as are involved in careful purification)

† The fact that an interaction is *kinetically* "strictly competitive" does not constitute proof that the competition is actually for the same site. In any instance where the interacting compounds are structurally unrelated such an interpretation should be considered with suspicion.

may result in partial desensitization of allosteric enzymes. It is therefore conceivable that a small spontaneously desensitized fraction may be responsible for the inhibition being incomplete.

With threonine-deaminase the inhibition is competitive and reaches 100%. None of the models could therefore be eliminated on this basis alone. If, however, on the strength of other evidence, model I proved inadequate also for threonine-deaminase, this enzyme would offer an example of an interesting limit-case, where the interaction, albeit not due to steric hindrance, is of such strength as to make the simultaneous binding of substrate and inhibitor (on the native enzyme) impossible.

We may now turn to another line of evidence. Since two systems of specific groups are involved in the enzyme–substrate and enzyme–(allosteric) effector complexes respectively, one expects to find two series of compounds able to complex with the protein, namely analogues of the substrate and analogues of the effector. The effector analogues, as well as the substrate analogues, should behave as strict competitive inhibitors according to model I. On the basis of models II and III, one may expect different effector analogues to behave in different ways:

(a) some analogues should behave like the natural allosteric inhibitor;

(b) others, able to displace the allosteric effector, while failing to interact with the substrate, should reactivate the inhibited enzyme while exerting no effect in the absence of inhibitor.

Both types of behaviour are observed with different analogues of isoleucine assayed for their effect upon the threonine-deaminase reaction (Changeux, 1962). For instance, norleucine strongly restores the activity when added in the presence of isoleucine. L-Leucine alone inhibits, and cooperates with isoleucine when added in its presence. These results are incompatible with model I, and prove that the inhibitory action of isoleucine on this enzyme, although "strictly competitive", cannot be due to binding at the active site.

The effects of valine are particularly interesting. When assayed alone at low concentrations of substrate, valine actually *activates* the reaction by increasing the affinity of the enzyme for threonine. Since valine is apparently an isoleucine analogue, one might believe that valine binds at the same site as isoleucine. However, when assayed in the presence of different concentrations of isoleucine, valine behaves as "partially competitive" towards the inhibitor, i.e. it reactivates the enzyme only to a finite value, which depends upon the isoleucine concentration (Fig. 3). These observations inevitably force the conclusion that threonine-deaminase bears not two, but at least three different sites; the active site, the isoleucine or "inhibitor" site and a valine or "activator" site. Binding of isoleucine at its site results in virtually abolishing the affinity of threonine for the active site, and the reverse must necessarily be true. Binding of valine at its site increases the affinity of the active site and simultaneously decreases the affinity of the isoleucine site.

Very similar observations have been made by Gerhart & Pardee (1962) with ATCase, where ATP acts both as an activator in its own right and as an antagonist of the inhibitor, GTP.

These complex "ternary" interactions would evidently be extremely difficult to account for by a direct interaction model, and we may conclude from this discussion of the kinetics of inhibition (and activation) of "controlling" enzymes of bacteria that the regulatory metabolites do not, in any case, act by steric hindrance (model I), and probably not by direct interaction (model II) between substrate, inhibitor and/or

activator. By elimination of other possible mechanisms, these findings constitute evidence in favour of the conclusion that the regulatory metabolites act indirectly by triggering an allosteric transition of the protein molecule.

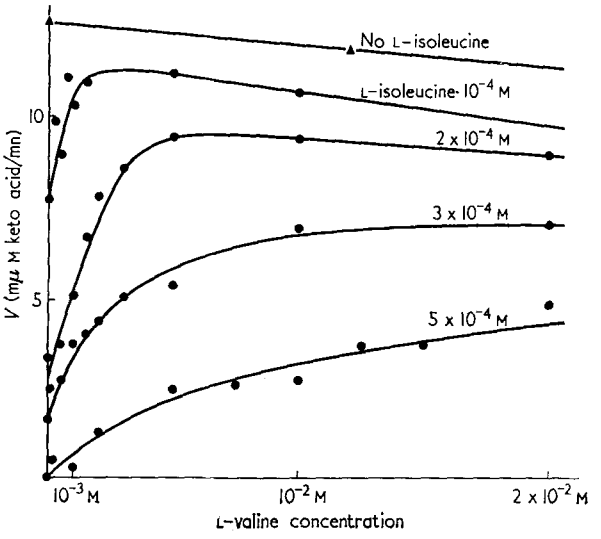

FIG. 3. Antagonistic effects of L-isoleucine and of L-valine on the activity of L-threonine-deaminase, in the presence of a constant concentration of L-threonine (2×10^{-2} M).

(b) *The "desensitization" effect*

We have already noted the fact that the sensitivity of regulatory enzymes to the inhibiting metabolite is, as a rule, an extremely labile property which may be lost as a result of various treatments, with little or no loss of activity. Complete "desensitization" has been obtained with threonine-deaminase (Changeux, 1961), ATCase (Gerhart & Pardee, 1962), homoserine-dehydrogenase (Patte *et al.*, 1962), PRPP-ATP-PPase (Martin, 1962), α-acetolactate synthetase (Martin & Cohen, unpublished results) in particular by treating with mercurials or urea and/or by gentle heating (cf. Fig. 4). Desensitization without loss of activity has also been observed as a result of mutations of the specific gene which controls the structure of threonine-deaminase (Changeux, unpublished results).

At first site, the simplest interpretation might appear to be that the desensitizing agents, or the mutations, destroy the inhibitor binding site itself. This interpretation is not satisfactory, however, because it does not account for the generality of the effect, nor for the exceptional lability of the sensitive state. In view of this, it seems far more likely that the action of the effector depends not only upon the integrity of its binding sites, but upon complete conservation of the native state. If this were the case, a slight disorganization of the protein as a whole (which might be brought about by a variety of attacks at different points on the molecule) would result in desensitization by uncoupling of the interaction without destroying the effector site or the active site. This interpretation has been validated by the important observation of Martin (1962) that desensitized PRPP-ATP-PPase still binds histidine, as tested by equilibrium dialysis. Similar tests have not yet been performed on any of the other systems discussed here, but certain observations of an entirely different kind made with threonine-deaminase and with ATCase lead to similar conclusions.

A remarkable feature common to both of these systems is that, at substrate concentrations below half saturation, the reaction velocity increases faster than the substrate concentration, while at low inhibitor concentrations the rate decreases faster than the inhibitor concentration; this means that the enzyme molecule can bind more than one substrate or one inhibitor molecule at a time and that in the *native* enzyme there is some sort of cooperative interaction between the homologous

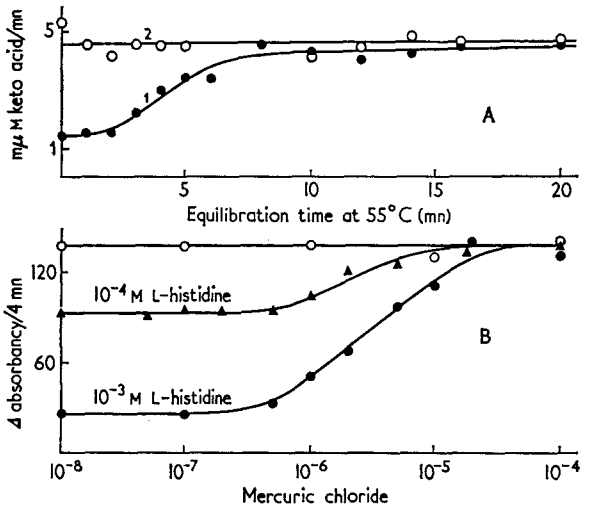

FIG. 4. (a) "Desensitization" of L-threonine-deaminase by heat treatment. *Curve* 1: activity measured with 2×10^{-2} M-L-threonine and 10^{-2} M-L-isoleucine. *Curve* 2: same L-threonine concentration, no L-isoleucine (after Changeux, 1961).

(b) "Desensitization" of PRPP-ATP-PPase by treatment with mercuric chloride (substrate added simultaneously with $HgCl_2$ at concentration indicated). *Upper curve*: enzyme assayed without addition of inhibitor. *Lower curves*: enzyme assayed at the two indicated concentrations of L-histidine (after Martin, 1962).

binding sites. These cooperative effects are closely comparable to the classical haem–haem interaction in haemoglobin to which we shall return later (Figs. 5 and 6). The striking fact which we wish to emphasize here is that the "desensitized" enzyme, in both cases, exhibits no trace of the *substrate cooperative effect*. As may be seen from Fig. 6 the kinetics of the reaction catalysed by desensitized ATCase are "normalized" and obey the Michaelis–Henri relation while, in the presence of native enzyme, the rate–concentration curve is sigmoid. Desensitization and "normalization" also exhibit a parallel dependence upon pH and ionic strength. Both the cooperative effect of substrate and the inhibitory effect of isoleucine on threonine-deaminase are maximal at pH values between 7 and 8, while both are abolished (reversibly) around pH 10. Finally, mutations which desensitize the enzyme also partially abolish or alter the cooperative effect of substrate.

These results show that both the cooperative interaction between substrate binding sites *and* the antagonistic interaction between inhibitor and substrate sites depend largely upon the same features of protein structure and are both similarly related to the integrity of the native state. The presence of several substrate and several inhibitor sites on each molecule of ATCase and threonine-deaminase actually suggests a more specific hypothesis as to which structures present in the native state may be

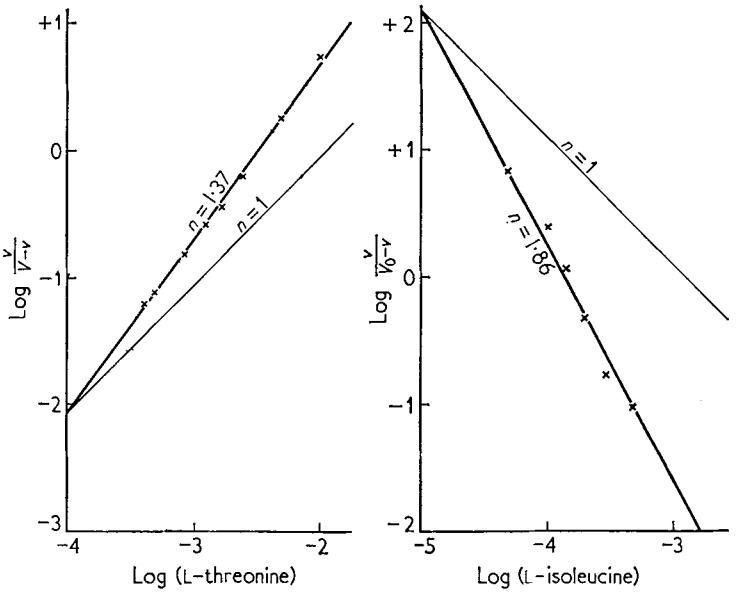

FIG. 5. L-threonine-deaminase activity as a function of (A) substrate and (B) allosteric inhibitor concentration. Both relations are seen to be conveniently represented by expressions of the form:

$$\log \frac{v}{V_{\max}-v} = n \log S - \log K \text{ (for substrate)}$$

$$\log \frac{v}{V_0-v} = \log K' - n' \log I \text{ (for inhibitor)}$$

These equations are formally identical with Hill's empirical relation for the binding of oxygen to haemoglobin. It is seen that for both substrate and inhibitor $2 > n > 1$ indicating cooperative interactions between homologous sites and also showing that the reaction is *not* truly bimolecular.

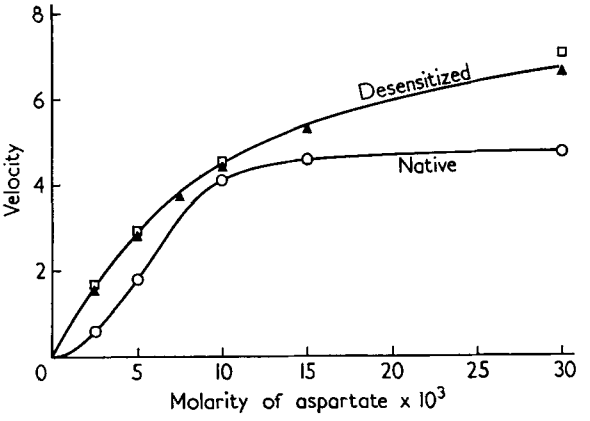

FIG. 6. Effects of desensitization of ATCase upon reaction kinetics. *Lower curve*: native enzyme, assayed in the absence of CTP. *Upper curve*: enzyme desensitized by heat treatment, assayed without inhibitor (squares) and with inhibitor (triangles) (CTP 2×10^{-4} M). (After Gerhart & Pardee, 1962.) Compare with Fig. 9.

primarily involved; namely that the native enzyme is made up of subunits and that the antagonistic as well as the cooperative interactions depend upon the relationships between these units. Desensitization might then result from alteration of these relations, i.e. from the rupture of bonds between the subunits; and this might of course apply also to systems which do not exhibit any substrate–substrate cooperation.

There is some fairly good, albeit incomplete, evidence in favour of this interpretation. Gerhart & Pardee (1962) have found that the sedimentation velocity of desensitized ATCase is decreased (from 11·6 to 5·9) by comparison with the native (sensitive) enzyme, and similar observations have been made by Patte *et al.* (1962) with homoserine-dehydrogenase. No detectable alterations of sedimentation velocity were observed, following desensitization, with PRPP-ATP-PPase nor with threonine-deaminase. The positive evidence of course carries more weight than the negative, especially since an incomplete separation of subunits need not entail a detectable alteration of the sedimentation velocity.

In any case we may conclude that the sum of the observations concerning the desensitization effect would be exceedingly difficult to reconcile with model II (not to mention model I) or more generally with any model which exclusively involves direct substrate–inhibitor interactions. On the other hand, the assumption that the interaction is due to an allosteric transition involving the protein molecule as a whole in its native state accounts very well for the characteristic lability of the sensitive state in these regulatory proteins and for the peculiar alteration of the kinetic parameters which attends desensitization of ATCase and threonine-deaminase.

3. Allosteric Effects as Conformational Alterations

Direct evidence of reversible conformational alterations provoked by the binding of a "regulatory" metabolite has been obtained with several proteins of higher organisms. We will discuss here only the best known and most significant systems, namely, beef liver glutamic-dehydrogenase, acetyl-CoA carboxylase from adipose tissue, muscle phosphorylase *b* and haemoglobin. Given the biochemical and physiological diversity of these systems, we will consider the properties of each of them in turn, reserving any general discussion for the next section.

As isolated in crystalline form from beef liver, the enzyme glutamic-dehydrogenase has a molecular weight of 10^6. The important discovery was made by Frieden (1959) some years ago that NADH provoked dissociation of the protein into subunits of molecular weight 250,000, while ADP antagonized the dissociation. Further work by Frieden (1961, 1962*a*,*b*,*c*), Yielding and Tomkins (1960, 1962), Tomkins, Yielding & Curran (1961), Tomkins & Yielding (1961) and Wolff (1962) has shown that this reversible dissociation is favoured or antagonized specifically by a somewhat bewildering variety of metabolites and also by non-specific agents, notably pH. The dissociative agents appear invariably to inhibit glutamic-dehydrogenase activity, while the associative agents exert the opposite effect. The great significance of this system as a model of physiological interactions at the molecular level was indicated in particular by the discovery that estrogens are among the most potent dissociative agents and that inhibition of glutamic-dehydrogenase is attended by concomitant *activation* of alanine-dehydrogenase. Tomkins *et al.* (1961) and Wolff (1962) later showed that thyroxine also is a potent inhibitor of glutamic-dehydrogenase, as well as a dissociative agent.

The specific inhibitors and the activators of glutamic-dehydrogenase include metabolites which are neither substrates nor coenzymes of GDH (estrogens, thyroxine, ADP, ATP, GDP, etc.) but the list also includes NADH, NAD^+, NADPH and $NADP^+$, which are coenzymes of the system, and the amino acids leucine, isoleucine and methionine, which may be considered as "secondary" substrates of the enzyme. This might seem to exclude the latter compounds from our initial, strictly limitative, definition of allosteric effectors as compounds which *do not* participate in the reaction. The definition, however, does not imply that one and the same compound cannot contribute both as an allosteric effector and as a participant of some kind in a reaction, but it does require that the two contributions should be distinct. The operational validity of this definition is in fact illustrated by the GDH system since, according to

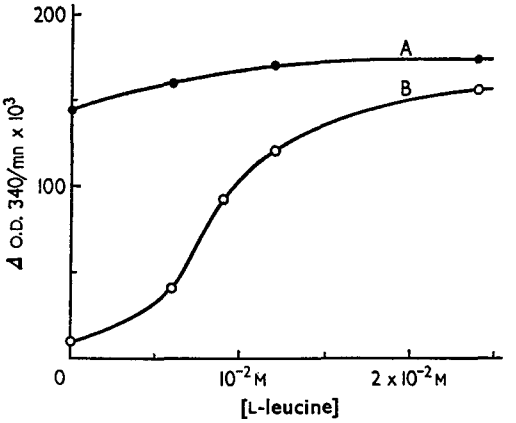

FIG. 7. Reversal by L-leucine of the inhibition of glutamic dehydrogenase by diethylstilbestrol. *Curve* A: no inhibitor. *Curve* B: enzyme assayed with $8{\cdot}0 \times 10^{-6}$ M-diethylstilbestrol. (After Tomkins & Yielding, 1961.)

Tomkins & Yielding (1961), the allosteric effect of leucine, for example, is due to binding at a site *other* than the active substrate site; while according to Frieden (1961) the dissociative effects of the reduced pyridine nucleotides are due to binding at sites distinct from the active coenzyme site. Moreover, glutamate itself has no effect on the dissociation. It is interesting to note that both the activation by leucine and the inactivation by diethylstilbestrol show evidence of cooperative (multimolecular) effects (Fig. 7). The similarity with ATCase, threonine-deaminase and other allosteric enzymes of bacteria is obvious. It is further strengthened by the finding that treatment of the enzyme with SH reagents renders it insensitive to diethylstilbestrol, and to ADP as well, without modifying the activity (Tomkins & Yielding, 1961).

By contrast with the bacterial systems, whose functions are simple and obvious, the physiological interpretation of the multiple sensitivities and activities of GDH appears exceedingly difficult. But while one may wonder whether each and all of the metabolites which act upon it *in vitro* have any significant role *in vivo*, it cannot be doubted that the complex allosteric reactivity of GDH does reflect its central, multivalent role in cellular metabolism. In any case the observations of Frieden and of Tomkins and his colleagues leave no doubt that the effectors which activate or inhibit the two potential activities of GDH act primarily by inducing a conformational alteration, eventually expressed as a dissociation of the protein.

Another remarkable example where a typical allosteric effect has been directly demonstrated to involve a conformational alteration has been provided by the recent work of Martin & Vagelos (1962) and Vagelos, Alberts & Martin (1962*a*,*b*). The enzyme is acetyl-CoA-carboxylase, which catalyses the two-stage reaction

$$\text{biotin E} + CO_2 + \text{ATP} \longrightarrow CO_2\text{-biotin E} + \text{ADP} + \text{P}$$
$$CO_2\text{-biotin E} + \text{acetyl-CoA} \longrightarrow \text{malonyl-CoA} + \text{biotin E}$$
$$\text{Sum: } CO_2 + \text{acetyl-CoA} + \text{ATP} \longrightarrow \text{malonyl-CoA} + \text{ADP} + \text{P}$$

It had been known for a long time that the biosynthesis of fatty acids is activated by citrate and it was assumed that citrate acted as a metabolic source of NADPH. When it was found that citrate specifically activates the enzyme acetyl-CoA-carboxylase, the metabolite was rather naturally believed to participate at some stage of the reaction itself. The exhaustive experiments of Vagelos *et al.* (1962*a*,*b*) have proved

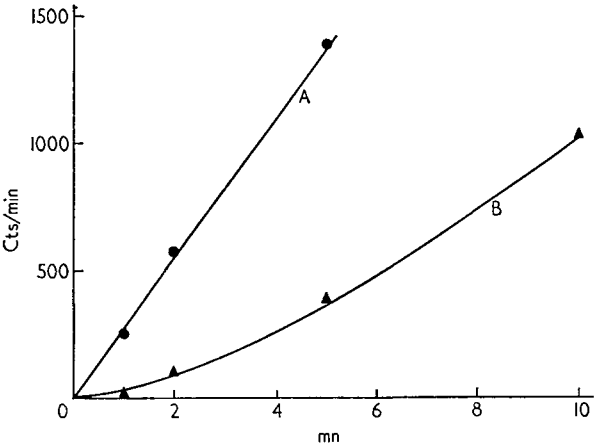

FIG. 8. Activation of acetyl-CoA-carboxylase by citrate. *Curve* A: citrate (5×10^{-3} M) added 30 min at 30°C before zero time. *Curve* B: citrate added at zero time (after Vagelos *et al.*, 1962*b*).

this assumption to be incorrect. No evidence was found that citrate participates in any way in the reaction. Fluorocitrate, as a matter of fact, is just as active as citrate itself, while other Krebs-cycle intermediates, except fumarate, are relatively inactive.† Since citrate modifies only the velocity of the reaction and not the substrate affinities, no direct interaction with substrate is apparently involved. The kinetics of the activation show that it is not immediate. Under the conditions of the experiment illustrated by Fig. 8, full activity is reached only after 30 minutes of incubation with citrate. By contrast, dilution of the activator brings about rapid inactivation of the enzyme. Centrifugation of the enzyme, in sucrose gradients, with and without previous incubation with citrate, revealed that activation is attended by an increase of sedimentation coefficient from 18 S to about 43 S. Although actual molecular weights have not yet been determined, it seems highly probable (in view particularly of the kinetics of activation and deactivation) that this large alteration of sedimentation coefficient is due to the formation of an active polymer (probably a trimer) from inactive monomers rather than to the folding up of the protein from an extended into a more globular form.

† Using a preparation from another source, Waite & Wakil (1962) have also found other Krebs-cycle intermediates to be active.

Probably the first allosteric enzyme mechanism to have been discovered and analysed in detail is the effect of 5′-AMP on muscle phosphorylase *b*. It was shown by Cori and his school (Cori, Colowick & Cori, 1938; Cori & Green, 1943; further references in Krebs & Fischer, 1962), already many years ago, that this enzyme which is almost inactive in the absence of 5′-AMP is instantaneously and reversibly activated in its presence. ADP, ATP and other nucleotides (except IMP) are inactive. It was naturally supposed at first that 5′-AMP played the role of a coenzyme. But further thorough experiments showed that the nucleotide does not participate in any detectable way in the reaction. Moreover, since the effector does not alter the affinity but only the velocity constant of the reaction, direct interaction with substrate (cf. model II above) appears improbable.

Now, as is well known, phosphorylase *b* may also be converted to an active state by an entirely different process, also discovered and analysed by the Cori school, namely phosphorylation by ATP (in the presence of a specific kinase) automatically attended by dimerization to phosphorylase *a*. This mechanism, by contrast with the 5′-AMP effect, is irreversible. Phosphorylase *a* is stable and reconversion of *a* to *b* follows a different course, namely hydrolytic dephosphorylation by a specific phosphatase.

This system therefore provides the proof that a reversible, presumably non-covalent, interaction between an allosteric effector and a protein may mimic, in part at least, the effects of an irreversible stable modification of protein structure involving initially a covalent reaction, followed by a reassociation of subunits. It seems inevitable to conclude that the transition induced by 5′-AMP in phosphorylase *b* is, in some respects at least, "equivalent" to the phosphorylation reaction. This conclusion is greatly strengthened by the demonstration by Kent, Krebs & Fischer (1958) that phosphorylase *b* dimerizes in the presence of 5′-AMP and also under suitable conditions crystallizes as such with the nucleotide (Fischer & Krebs, 1958; Kent *et al.*, 1958). It should be added that phosphorylase *b* is known to be made of two subunits, hence phosphorylase *a* of four; and it has been found that, by treatment with *p*-chloromercuribenzene sulfonate, phosphorylases *a* and *b* dissociate into inactive subunits (Madsen & Cori, 1956). There is little doubt therefore that the activity of phosphorylase is dependent upon its "quaternary" structure and that certain acid (thiol) groups play a critical role in maintaining this structure. Nor is there any doubt that the activation of phosphorylase *b* by AMP results from a conformational alteration. However, a very significant question remains to be solved, namely whether this alteration is induced *directly* by the binding of the nucleotide, the dimerization reaction being then a result of this primary effect, or whether the activating alteration results from the dimerization itself.

All the examples which we have considered until now relate to enzymic proteins. Haemoglobin is an example of a non-enzymic protein whose specific regulatory competence has long been recognized. As is well known, the dissociation curve of oxyhaemoglobin as a function of oxygen tension is sigmoid, demonstrating a cooperative effect of the binding sites. By contrast, in the case of myoglobin the dissociation function is a simple adsorption isotherm (i.e. identical to the Michaelis–Henri relation). When the two curves are plotted on the same graph (Fig. 9), the analogy between this situation and that of native (sensitive) and desensitized threonine-deaminase or ATCase is obvious. This functional difference between haemoglobin and myoglobin is of course known to depend upon the tetrameric structure of the former and the monomeric state of the latter.

As is also well known, haemoglobin is subject to another effect endowed with regulatory significance, namely the Bohr effect, which consists of an increase of the oxygen dissociation as the pH is lowered (i.e. *in vivo* as CO_2 tension increases). Wyman (1947) demonstrated several years ago that the Bohr effect is due to a discharge of protons provoked by the binding of oxygen. The recent work of Riggs (1959) seems to identify the acidic groups responsible for the Bohr effect with cysteinyl residues which are apparently also involved in the haem–haem interaction, but it is also possible that the actual "oxygen-linked" acid groups are imidazole residues, presumably closely associated with the thiol groups (Benesch & Benesch, 1961). In any case, the blocking of the latter groups (by mercurials or N-ethyl maleimide) alters both the Bohr effect and the haem–haem interaction, just as similar treatments have been found to abolish allosteric effects in bacterial and other regulatory enzymes.

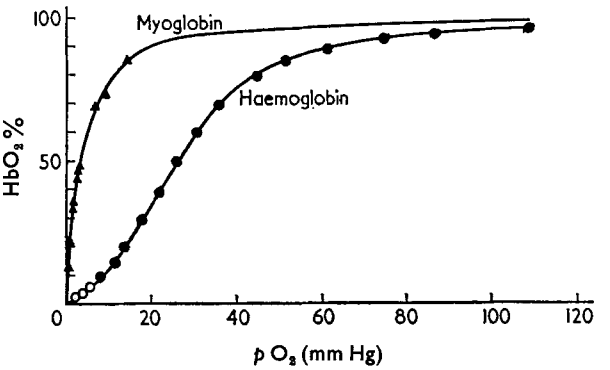

FIG. 9. Oxygen dissociation curves of human haemoglobin (data from Morgan & Chichester, 1935) and of horse heart myoglobin (data from Theorell, 1934). Compare with Fig. 6.

The interactions may also be altered as a result of mutations, as in the case of threonine-deaminase. In haemoglobin H, both the Bohr effect and the haem–haem interactions are abolished. This is particularly interesting, since this haemoglobin contains only β chains: it suggests that α–β chain inter-relations play an important role in both effects (Benesch, Ranney, Benesch & Smith, 1961).

It was believed for a long time that the cooperative binding of oxygen molecules on haemoglobin was due to direct interactions (of the kind shown by model II) between haem groups, presumed to lie very close to one another in the protein. The work of Perutz *et al.* (1960) has demonstrated that the four haem groups actually are wide apart, excluding any possibility of direct interaction and imposing the only alternative interpretation, namely that the interaction is indirect, therefore presumably due to a conformational alteration. As a matter of fact, it had been known for several years that oxyhaemoglobin and reduced haemoglobin occur in different crystal forms (Haurowitz, 1938). The recent crystallographic work of Muirhead & Perutz (personal communication) has indicated that the *distance* between certain SH residues in the molecule may be shifted by about 19% upon oxygenation, providing direct though still tentative evidence of a conformational alteration. Thus, in the case of haemoglobin, there is complete evidence that the regulatory effect, i.e. the cooperative binding of oxygen, is related to a reversible, discrete conformational alteration of the protein, i.e. in our nomenclature, to an allosteric transition. Actually,

thanks to the considerable work which has been devoted to it, the haemoglobin system provides the most valuable model from which to start in the further analysis and interpretation of allosteric effects in general.

4. General Discussion and Conclusions

(a) *Validity, qualifications and limitations of the allosteric model*

We may now reconsider the general model proposed in the Introduction for the functional structures of "controlling" proteins. Our aim should be to inquire whether, in the light of the experimental evidence analysed in the preceding sections, the allosteric model appears to be valid and whether it could be further specified and qualified.

In its most general form the allosteric mechanism is defined by two statements, one negative, the other positive.

1. No direct interactions of any kind need occur between the substrate(s) of an allosteric protein and the regulatory metabolite which controls its activity.

2. The effect is *entirely due* to a reversible conformational alteration induced in the protein when it binds the specific effector.

These two statements are not independent. Besides its biological significance (which we shall discuss below), the first of these statements is essential because, if and where it can be proved correct, the second statement must also be correct. Conversely, in those cases where the first statement is inadequate or unproved the second one could hardly ever be proved, even if direct evidence of a conformational alteration were obtained.

As we have seen, direct evidence that the action of a regulatory metabolite involves a conformational alteration of protein structure is available only in four cases (GDH, acetyl-CoA-carboxylase, phosphorylase *a* and haemoglobin). In one at least of these instances (haemoglobin) "effector" and "substrate" are one and the same molecule. It should perhaps be emphasized again that this does not invalidate the allosteric model, provided the substrate function and the allosteric effector function can be operationally distinguished. This is certainly the case for haemoglobin, and also for the effects of leucine and of NADPH on glutamic-dehydrogenase.

Most of the evidence available at present concerns the structural specificity and the kinetics of action of regulatory metabolites upon certain enzymes. This evidence can be used to test the validity of the first statement, but since this statement is a strictly negative one the evidence also can only be negative. Putting it more precisely: the allosteric model is compatible with virtually any kinetics while models involving direct interactions at the active site are more restrictive. In spite of this logical difficulty, the bulk of the evidence (concerning in particular the bacterial systems) seems to be overwhelmingly in favour of the allosteric model, because neither the chemical properties, nor the structural specificity, nor the kinetics of action of the regulatory metabolites appear compatible with "direct interaction" models.

In addition, the generality of the desensitization effect, which is a positive prediction of the allosteric model, is evidently exceedingly difficult to account for by a direct interaction model. The occurrence of desensitized states of a regulatory enzyme (whether as a result of mutation, or of the action of denaturing agents) therefore constitutes in any given instance one of the most specific tests of the validity of the

allosteric model and may be considered to prove it when (as is the case in several systems which we discussed) the other evidence independently points to the same conclusion.

This statement should, however, be qualified by carefully defining the operational meaning of the expression "allosteric transition". Throughout the preceding discussion, we have treated it as equivalent to "specifically inducible conformational alteration of protein structure". However, the only conclusion which can be drawn from kinetic data together with the occurrence of desensitization is that a given effect is indirect, due to the binding of substrate and effector at sites remote from each other and whose interaction must therefore be mediated through the protein. Such mediation would not necessarily involve a conformational alteration *sensu stricto*. It might conceivably be due, for instance, to a redistribution of charge within the molecule without *detectable* alteration of its spatial configuration. In a protein molecule, however, any redistribution of charge might be expected to involve or to facilitate a true conformational alteration. Actually, as we have seen, indirect evidence suggests in many cases and direct observations prove in a few instances that allosteric transitions involve the breaking, or formation, or substitution of bonds between subunits in the protein. Whether this may be considered a general rule is evidently a question of great importance, which might perhaps profitably be stated also in the following way: do allosteric transitions occur in monomeric proteins containing a single polypeptide chain? This problem is related to a more general one, which is the role of quaternary structures in the biological activity of proteins. Following the discovery by Cori of the phosphorylase conversions it has become increasingly evident during the past few years that many proteins, particularly enzymes, are homo- or heteropolymers, and that their activity is dependent upon correct association between their subunits (cf. Lwoff & Lwoff, 1962). In any such protein, disorientation or re-orientation, however slight, of the subunits with respect to each other would entail loss or gain of activity. Given these facts and the evidence which we have discussed here concerning regulatory proteins, one may feel that more complete observations, once available, might justify the conclusion that allosteric transitions frequently involve alterations of quaternary structure.

Even if this assumption were generally valid, the role of the effector itself would remain to be accounted for. In the best studied and also probably the simplest case, haemoglobin, the role of the effector-substrate, oxygen, in inducing the transition is far from being completely understood. It is certain, however, that the binding of oxygen to a haem induces within the molecule a redistribution of charge, expressed as a discharge of protons by an acidic group; hence *motu contrario* the pH effect on oxygen affinity. Similar pH effects have been observed, as we already noted, with several other allosteric systems. In the case of threonine-deaminase both the positive interaction between active sites and the negative interaction between active and allosteric sites are abolished at high pH, suggesting that the allosteric transition ultimately depends upon the ionization of certain critical acid groups (or their conjugate base). In addition, let us recall the fact that in most systems the allosteric effect is blocked by reagents known to attack certain acidic groups (thiol and imidazole), also suggesting that such groups play a critical role in the transition. It should be clear, however, that the effect could not be ascribed solely or primarily to the charge or polarity of the effector itself, but only to the specific type of bonding which it forms with the protein. Again consider the case of threonine-deaminase, where valine

increases and isoleucine decreases the affinity of the active site, although both amino acids carry the same charge with the same absence of polarity in their side-chain.

No contradiction need be seen between the extreme specificity of these effects and the fact that similar or identical transitions of structure may *also* result in certain systems from the action of non-specific agents (cf. glutamic-dehydrogenase). It would evidently be very misleading to consider reversible discrete conformational alterations, attended by modifications of biological activity, as a privilege of regulatory proteins. There is of course ample experimental evidence showing that such reversible alterations of structure occur in many proteins under the action of non-specific conditions or chemical agents, including in particular pH, ionic strength, hydrogen-bonding or hydrophobic compounds, and mercurials. The essential properties of typical regulatory proteins (i.e. the capacity to undergo an allosteric transition triggered by the stereospecific binding of a particular metabolite) are to be understood as highly specialized manifestations of general properties, shared by all or most proteins. In other words, an allosteric protein represents the outcome of a process of selective development of a molecular species where the flexibility of protein structure assumes the specific functional role of mediating certain chemical signals.

The "induced-fit" theory of enzyme action, proposed by Koshland (1958, 1960), involved the following postulate:

(a) a precise orientation of catalytic groups is required for enzyme action;

(b) the substrate may cause an appreciable change in the three-dimensional relationship of the amino acids at the active site;

(c) the changes in protein structure caused by a substrate will bring the catalytic groups into proper orientation for reaction, whereas a non substrate will not.

While the purpose of this model is to account for certain anomalous features of enzyme specificity, its central postulate is similar to the basic assumption of the allosteric model, to the extent that it invokes a functional role for the flexibility of protein structure. The evidence concerning allosteric systems shows that the binding of substrate (or coenzyme) does provoke conformational alterations in certain proteins. Such observations must be interpreted with caution, however, since as we have seen in the case of glutamic-dehydrogenase the allosteric sites for leucine and NADPH are distinct from the active sites, and the substrate itself, glutamate, does not affect the dissociation. On the other hand, in the case of ATCase and threonine-deaminase (actually in *any* case where the allosteric effect results in a decrease of substrate affinity) the substrates must be considered to provoke a transition *opposed* to the transition corresponding to the binding of inhibitor. One is tempted to suggest that in those cases where the binding of substrate does provoke a detectable conformational alteration of an enzyme the effect may often turn out to be interpretable in terms of a regulatory allosteric transition. The possibility that the "induced-fit" model might be extended to involve regulatory effects has in fact been mentioned by Koshland himself in a recent theoretical paper (Koshland, 1962).

(b) *The biological significance of allosteric control systems*

Even granting that allosteric mechanisms exist and intervene at many stages of cellular metabolism it might be asked whether one would be justified in considering that this particular class of interactions plays a special, uniquely significant role in the control of living systems.

Other types of mechanisms contribute to cellular regulation. We need only mention mass action; while it inevitably intervenes, a living system is constantly fighting against, rather than relying upon, thermodynamic equilibration. The thermodynamic significance of specific cellular control systems precisely is that they successfully circumvent thermodynamic equilibration (until the organism dies, at least). An illustration of this statement is given by certain metabolic pathways which are both thermodynamically and physiologically reversible, such as the synthesis of glycogen from glucose-1-phosphate. It is now established that the cells do not use the same pathways for synthesis and degradation of glycogen, and that each of these pathways is submitted to different specific controls, involving hormones and other metabolites, none of which participate directly in the reactions themselves (cf. Krebs & Fischer, 1962; Rall & Sutherland, 1961; Leloir, 1961); all this evidently because the physiological requirements could not be satisfied otherwise, certainly not by simply obeying mass action.

Competition between enzymes for common substrates evidently plays a role in the balance of metabolism by distributing certain important metabolites, such as coenzymes, between different pathways. Such mechanisms, however, would by themselves be unable to govern and control, that is to say to *modify*, the distribution of building blocks or chemical potential according to the requirements of remote pathways, or to chemical alterations of the environment, or to the physiological meaning of chemical signals issued by other cells. For the chemical activities of a cell to be precisely adjusted to its own requirements, adapted to the environment and directed towards the performance of a particular function, the specific activity of those proteins which are responsible for critical metabolic steps must be altered electively in response to the presence of certain metabolites playing the role, not of substrates for the reaction in question, but of chemical signals.

The primary reason for considering allosteric proteins as essential and characteristic constituents of biochemical control systems is their capacity to respond immediately and reversibly to specific chemical signals, effectors, *which may be totally unrelated to their own substrates, coenzymes or products.*

We have discussed several examples which illustrated this point (and need not be recalled here), leading us to the paradoxical conclusion that the structure and *sui generis* reactivity of an allosteric effector is "irrelevant" to the interpretation of its effects. There remains no real chemical paradox, once it is recognized that an allosteric effect is indirect, being mediated entirely by the protein and due to a specific transition of its structure. Still, the arbitrariness, chemically speaking, of certain allosteric effects appears almost shocking at first sight, but it is this very arbitrariness which confers upon them a unique physiological significance, and the biological interpretation of the apparent paradox is obvious. The specific structure of any enzyme-protein is of course a pure product of selection, necessarily limited, however, by the structure and chemical properties of the actual reactants. No selective pressure, however strong, could build an enzyme able to activate a chemically impossible reaction. In the construction of an allosteric protein this limitation is abolished, since the effector does not react or interact directly with the substrates or products of the reaction but only with the protein itself. A regulatory allosteric protein therefore is to be considered as a specialized product of selective engineering, allowing an indirect interaction, positive or negative, to take place between metabolites which otherwise would not or even could not interact in any way, thus eventually bringing a particular reaction

under the control of a chemically foreign or indifferent compound. In this way it is possible to understand how, by selection of adequate allosteric protein structures, any physiologically useful controlling connection between any pathways in a cell or any tissues in an organism may have become established. It is hardly necessary to point out that the integrated chemical functioning of a cell requires that such controlling systems should exist. The important point for our present discussion is that these circuits of control could not operate, i.e. could not have evolved, if their elementary mechanisms had been restricted to direct chemical interactions (including direct interactions *on* an enzyme site) between different pathways. By using certain proteins not only as catalysts or transporters but as molecular receivers and transducers of chemical signals, freedom is gained from otherwise insuperable chemical constraints, allowing selection to develop and interconnect the immensely complex circuitry of living organisms. It is in this sense that allosteric interactions are to be recognized as the most characteristic and essential components of cellular control systems.

This brings up the problem of hormones as allosteric effectors; we are now referring mostly to hormones of small molecular weight. The specificity of hormones; their capacity of simultaneously activating or inhibiting a variety of metabolic processes and of exerting different effects on different tissues; the surprisingly small number of reactions in which they have been proved to take part as reactants opposed to the large number of enzymes upon which they have been found to act; the lack of chemical reactivity of certain hormones, such as the steroids; all in fact of these physiologically essential and chemically bewildering properties could be accounted for by the assumption that hormones in general (but not necessarily in all of their manifestations) act as allosteric effectors, each of them able specifically to trigger allosteric transitions in a variety of different proteins, mostly enzymes, but possibly also genetic repressors. In fact it seems difficult to imagine any biochemical mechanism other than allosteric which could allow a single chemical signal to be understood and interpreted simultaneously in different ways by entirely different systems; although this appears to be the case for many hormones.

Unfortunately, glutamic-dehydrogenase is one of the very few enzyme systems where hormones (thyroxine and estrogens) have undoubtedly been proved to act as allosteric effectors and we shall resist the temptation to make sweeping generalizations. The most serious objection to the concept of allosteric control is that it *could* be used to "explain away" almost any mysterious physiological mechanism.

While the possession of this universal key raises serious latent dangers for the experimenter, it is of such value to living beings that natural selection must have used it to the limit. Structural mutations occurring in a "classical" (non-regulatory) enzyme may alter one or both of its kinetic constants with respect to its normal substrate. Mutations occurring in an allosteric protein may modify its functional properties in a much larger number of ways. Figure 10 illustrates the properties of a number of mutants of the gene which determines the structure of threonine-deaminase in *E. coli*. As it may be seen, some of the mutant proteins have partially lost their sensitivity to isoleucine, others have increased it; the shape of the inhibition curves are different, indicating that the degree of cooperation between allosteric sites has been altered, as well as their interactions with the active sites. How these exquisite possibilities may be used for adjusting allosteric systems precisely to their functions is illustrated by Fig. 11 taken from a paper by Riggs (1959) which shows that the Bohr effect exhibited by haemoglobins of different mammals is closely correlated to their size.

In the present paper, we have exclusively discussed allosteric systems which control the *activity* of enzymes or other proteins. It is of extreme interest to enquire whether allosteric effects may also be involved in the specific control of protein *synthesis*, i.e. in the mechanisms of "genetic repression".

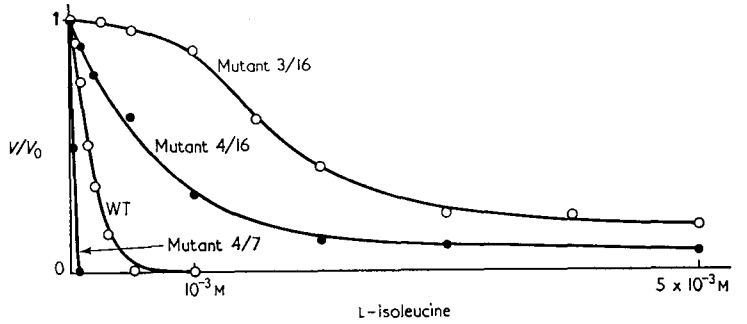

FIG. 10. Inhibition by L-isoleucine of various structural mutants of L-threonine-deaminase.

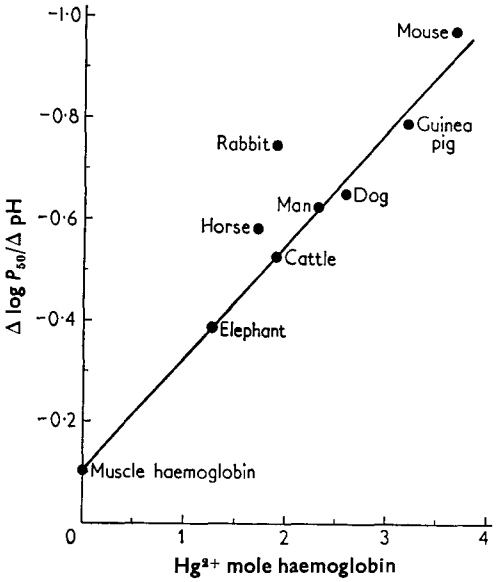

FIG. 11. Magnitude of the Bohr effect with haemoglobin from different mammals correlated with the number of Hg atoms taken up per molecule of protein (after Riggs, 1959).

Let us briefly recall the organization of this control system as it is understood today (see Fig. 12). The structural information written as a sequence of deoxyribonucleotides in a gene is first transcribed into a ribonucleotide sequence, the messenger. The messenger attaches to a ribosome, where the transcription into a polypeptide sequence takes place, the amino acids being transferred over from amino acyl S-RNA and positioned along the sequence by appropriate base-pairing between messenger and S-RNA.

This system is controlled at the level of messenger synthesis by specific agents, the repressors, able to recognize and bind electively certain genetic loci, called operators, which apparently function as exclusive initiation points for the first transcription.

The DNA segment whose transcription is thus "coordinated" by a given operator may involve one or several genes (or cistrons); it constitutes a unit of genetic expression called an operon. The synthesis of the corresponding protein(s) is therefore governed by the homologous repressor which, in turn, is synthesized under the control of a specialized "regulator" gene. In most, if not all cases, the activity of the repressor, i.e. presumably its ability to bind the corresponding operator, is controlled

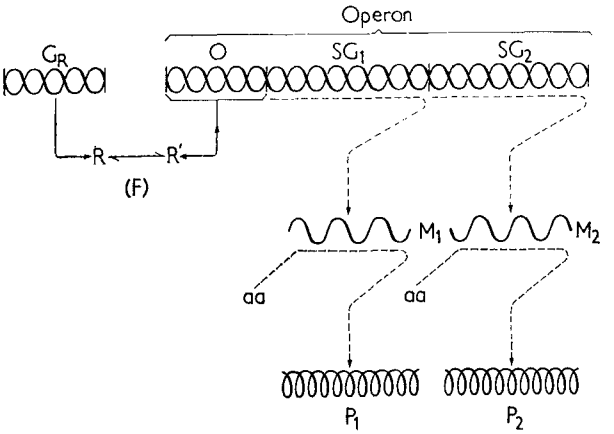

FIG. 12. General model of protein synthesis controlled by genetic repression (Jacob & Monod, 1961).

by specific small molecular compounds acting either as positive effectors (activating the repressor and thereby blocking messenger and protein synthesis) or as negative effectors (inhibiting the repressor and thereby inducing the synthesis of the messenger and of the protein(s)). The positive repression effectors are called "co-repressors". The negative repression effectors are called "inducers" (Jacob & Monod, 1961; Monod, Jacob & Gros, 1961).

We are interested here exclusively in the nature of the inducer–repressor–operator interaction. It was still considered likely not long ago that the repressor might be a polyribonucleotide (Jacob & Monod, 1961). This assumption, which did not by itself account for the repressor–inducer interaction (Jacob, Sussman & Monod, 1962), has met with further serious difficulties, while several lines of indirect experimental evidence suggest that the active product of a regulator gene is a protein, present in exceedingly minute amounts in cells. Since it has not proved possible so far to isolate a repressor and to observe its interactions *in vitro*, what knowledge we have comes from *in vivo* experiments, which can, however, be conducted under rigorous conditions, excluding many complications and ambiguities. On this basis, the following conclusions may be considered established.

1. The stereospecificity of the interaction is extreme (Monod, Cohen-Bazire & Cohn, 1951).

2. The interaction is virtually immediate and reversible, being completed both ways in less than 15 seconds according to the recent elegant work of Képès (1962).

3. The genetic–biochemical evidence shows that a single gene, therefore presumably a single specific macromolecular constituent, the repressor, is responsible for the specificity of the interaction (cf. Jacob & Monod, 1962).

4. Single mutations of this gene abolish the repressor–effector interaction while conserving the repressor–operator affinity (Willson, Perrin, Jacob & Monod, 1963).

It is evident that all these properties are immediately accounted for if the repressor is an allosteric protein possessing two sites, one of which binds the operator, the other the (positive or negative) effector. Almost any other model, by contrast, meets with extreme difficulties which need not be gone into here.

There are therefore strong reasons to assume as a working hypothesis that the specific effects of small molecules in activating or inhibiting, at the genetic level, the synthesis of messenger RNA and protein are mediated by an allosteric transition of the repressor.

For the time being only a few, perhaps a dozen, regulatory proteins have clearly been shown to exert their function by virtue of undergoing an allosteric transition. All of these proteins (except haemoglobin) are metabolic enzymes. One may rather confidently expect the number of metabolic control systems experimentally identified as allosteric mechanisms to increase considerably in the next few years. If, as one may hope, genetic repressors can eventually also be isolated and directly tested as to their indirectly inferred properties, it may be found that the fundamental elements or biological control systems, whether governing the activity or the synthesis of specific macromolecules, are allosteric proteins, those most elaborate products of molecular evolution.

Note added in proof.

With respect to the problem of the correlation between allosteric effects and association–dissociation reactions of proteins, two recent observations should be mentioned:

Frieden (manuscript in preparation) has found that the molecular weight of GDH at high dilutions (such as are used for assay of enzyme activity) is 250,000. Therefore no direct and immediate correlation appears to exist between the state of aggregation of this enzyme and its activity. Similarly, the effect of AMP on the state of aggregation of phosphorylase *b* has been studied at protein concentrations corresponding to those used for enzyme assay. Centrifugation in sucrose gradients showed that the sedimentation velocity was the same both in the presence and in the absence of AMP (Ullmann, Vagelos & Monod, unpublished). It would appear therefore that the activation of phosphorylase *b* by AMP does not directly depend upon dimerization of the protein. Thus while allosteric agents frequently appear to affect the state of aggregation of the sensitive proteins, the activating or inhibitory effects of the same agents do not seem necessarily to depend upon the association–dissociation reaction itself. The nature of the indirect correlation which appears, nevertheless, to exist between the two classes of effects remains to be explored and interpreted.

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REFERENCES

Benesch, R. & Benesch, R. E. (1961). *J. Biol. Chem.* **236**, 405.
Benesch, R. E., Ranney, H. M., Benesch, R. & Smith, G. M. (1961). *J. Biol. Chem.* **236**, 2926.
Changeux, J. P. (1961). *Cold Spr. Harb. Symp. Quant. Biol.* **26**, 313.
Changeux, J. P. (1962). *J. Mol. Biol.* **4**, 220.
Cori, G. T., Colowick, S. P. & Cori, C. F. (1938). *J. Biol. Chem.* **123**, 381.
Cori, G. T. & Green, A. A. (1943). *J. Biol. Chem.* **151**, 31.
Dische, Z. (1941). *Bull. Soc. Chim. Biol.* **23**, 1140.

Fischer, E. H. & Krebs, E. G. (1958). *J. Biol. Chem.* **231**, 65.
Frieden, C. (1959). *J. Biol. Chem.* **234**, 809.
Frieden, C. (1961). *Biochim. biophys. Acta*, **47**, 428.
Frieden, C. (1962*a*). *Biochim. biophys. Acta*, **62**, 421.
Frieden, C. (1962*b*). *Biochim. biophys. Acta*, **59**, 484.
Frieden, C. (1962*c*). *J. Biol. Chem.* **237**, 2396.
Gerhart, J. C. & Pardee, A. B. (1961). *Fed. Proc.* **20**, 224.
Gerhart, J. C. & Pardee, A. B. (1962). *J. Biol. Chem.* **237**, 891.
Haurowitz, F. (1938). *Z. Physiol. Chem.* **254**, 268.
Jacob, F. & Monod, J. (1961). *J. Mol. Biol.* **3**, 318.
Jacob, F. & Monod, J. (1962). In *Cytodifferentiation and Macromolecular Synthesis*, 21st Growth Symposium. New York: Academic Press Inc., in the press.
Jacob, F., Sussman, R. & Monod, J. (1962). *C.R. Acad. Sci., Paris*, **254**, 214.
Kent, A. B., Krebs, E. G. & Fischer, E. H. (1958). *J. Biol. Chem.* **232**, 549.
Képès, A. (1962). *Biochim. biophys. Acta*, in the press.
Koshland, D. E. (1958). *Proc. Nat. Acad. Sci., Wash.* **44**, 98.
Koshland, D. E. (1960). *Advanc. Enzymol.* **22**, 45.
Koshland, D. E. (1962). In *Horizons in Biochemistry*, p. 265. New York: Academic Press Inc.
Krebs, E. G. & Fischer, E. H. (1962). *Advanc. Enzymol.* **24**, 263.
Leloir, L. F. (1961). *Harvey Lect.* ser. **56**, 23.
Lwoff, A. & Lwoff, M. (1962). *J. Theoret. Biol.* **2**, 48.
Madsen, N. B. & Cori, C. F. (1956). *J. Biol. Chem.* **223**, 1055.
Martin, D. B. & Vagelos, P. R. (1962). *J. Biol. Chem.* **237**, 1787.
Martin, R. G. (1962). *J. Biol. Chem.* **237**, in the press.
Monod, J., Cohen-Bazire, G. & Cohn, M. (1951). *Biochim. biophys. Acta*, **7**, 585.
Monod, J. & Jacob, F. (1961). *Cold Spr. Harb. Symp. Quant. Biol.* **26**, 389.
Monod, J., Jacob, F. & Gros, F. (1961). *Biochem. Soc. Symp.* **21**, 104.
Morgan, V. E. & Chichester, D. F. (1935). *J. Biol. Chem.* **110**, 285.
Novick, A. & Szilard, L. (1954). In *Dynamics of Growth Processes*. Princeton: Univ. Press, 21.
Patte, J. C., Le Bras, G., Loviny, T. & Cohen, G. N. (1962). *Biochim. biophys. Acta*, in the press.
Perutz, M. F., Rossmann, M. G., Cullis, A. F., Muirhead, H., Will, G. & North, A. C. T. (1960). *Nature*, **185**, 416.
Rall, T. W. & Sutherland, E. W. (1961). *Cold Spr. Harb. Symp. Quant. Biol.* **26**, 347.
Riggs, A. (1959). *Nature*, **183**, 1037.
Stadtman, E. R., Cohen, G. N., Le Bras, G. & de Robichon-Szulmajster, H. (1961). *J. Biol. Chem.* **236**, 2033.
Theorell, H. (1934). *Biochem. Z.* **268**, 73.
Tomkins, G. M. & Yielding, K. L. (1961). *Cold Spr. Harb. Symp. Quant. Biol.* **26**, 331.
Tomkins, G. M., Yielding, K. L. & Curran, J. (1961). *Proc. Nat. Acad. Sci., Wash.* **47**, 270.
Umbarger, H. E. (1956). *Science*, **123**, 848.
Umbarger, H. E. & Brown, B. (1957). *J. Bact.* **73**, 105.
Umbarger, H. E. & Brown, B. (1958). *J. Biol. Chem.* **233**, 415.
Vagelos, P. R., Alberts, A. W. & Martin, D. B. (1962*a*). *Biochem. Biophys. Res. Comm.* **8**, 4.
Vagelos, P. R., Alberts, A. W. & Martin, D. B. (1962*b*). *J. Biol. Chem.* in the press.
Waite, M. & Wakil, S. J. (1962). *J. Biol. Chem.* **237**, 2750.
Willson, C., Perrin, D., Jacob, F. & Monod, J. (1963). *Biochem. Biophys. Res. Comm.* in the press.
Wolff, J. (1962). *J. Biol. Chem.* **237**, 230.
Wyman, J. (1947). *Advanc. Protein Chem.* **4**, 420.
Yates, R. A. & Pardee, A. B. (1956). *J. Biol. Chem.* **221**, 757.
Yielding, K. L. & Tomkins, G. M. (1960). *Proc. Nat. Acad. Sci., Wash.* **46**, 1483.
Yielding, K. L. & Tomkins, G. M. (1962). *Biochim. biophys. Acta*, **62**, 327.