† This author, as a visiting investigator at the Institute for Muscle Research, was aided in part by a grant from the Muscular Dystrophy Associations of America, Inc., and by a grant from the National Institutes of Health (RG-4643).

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[23] Leonard I. Grossweiner and Max S. Matheson (*J. Chem. Phys.*, **20**, 1654–1655, 1952; **22**, 1514–1526, 1954) have reported observing an X-ray-induced fluorescence from ice at low temperatures. The emission at −183° C. had an almost symmetrical band with a peak at 385 μ. They observed no phosphorescence whatever. That energy was trapped, however, was made evident from glow-curve studies following X-ray excitation. The difference between their activation energy (0.25 ev.) and ours (0.02 ev.) indicates that different mechanisms are involved in "trapping" the energy. This would be expected, since energy was stored in the one instance by X-ray excitation and in the other instance by ultraviolet light. We have made no study of the influence of oxygen on the emission, and dissolved oxygen was not excluded prior to freezing. Grossweiner and Matheson observed a suppression of thermoluminescence by oxygen.

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*REPRESSED AND INDUCED ENZYME FORMATION: A UNIFIED HYPOTHESIS**

BY HENRY J. VOGEL

DEPARTMENT OF MICROBIOLOGY, YALE UNIVERSITY

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A specific antagonism, termed "enzyme repression," of the formation of the biosynthetic enzyme acetylornithinase in *Escherichia coli* has recently been described.[1] The repressing agent was found to be arginine (or a substance related to arginine), which is the "end product" of the biosynthetic sequence involved. Enzyme repression by an exogenously available end product (combined with

anabolic utilization of this product) represents a highly effective regulatory device, since it permits the cell involved to curtail superfluous enzyme production. Enzyme repression by an endogenously produced substance can provide a feed-back arrangement that governs, in line with cellular demands, the flow of metabolites through the pathway leading to this substance. Accordingly, enzyme repression is a control mechanism that complements enzyme induction: in either case, the cell tends to form enzymes when they are needed and tends not to form enzymes when they are not needed. It has been pointed out that repressibility appears to be a widespread property of enzyme-forming systems, which presumably was positively selected in the course of evolution. The possibility that enzyme repression plays a role in the development or differentiation of higher forms of life has also been briefly mentioned. The present communication is concerned with a hypothesis of enzyme induction and repression.

"Small-Molecule" Control of Enzyme Formation.—As previously discussed, repression and induction appear to be separable from the process of enzyme formation per se; in particular, there are good grounds for the view that induction is not a *sine qua non* of enzyme formation. It is for this reason that repression and induction are best pictured as control mechanisms rather than as inherent features of enzyme biogenesis.

The following small-molecule control situations with respect to enzyme formation are conceivable: (i) indifferent enzyme synthesis (no control); (ii) induced enzyme synthesis; and (iii) repressed enzyme synthesis. One and the same enzyme-forming system may be both inducible and repressible,[2] or may be neither. Again, a given enzyme-forming system may be inducible but not repressible, or repressible but not inducible.

Repression and induction can thus be considered to have a functional correspondence. In addition, these processes may well depend on corresponding molecular mechanisms. The question then arises how a relatively small molecule, such as an inducer or a represser, can specifically modify the formation of a particular protein of large molecular weight.

A Unified Hypothesis of Induction and Repression.—From their relative specificity it seems reasonable to assume that induction or repression depend, respectively, on an interaction between inducer or represser (or their "active" derivatives), on the one hand, and a macromolecule, presumably of template nature, on the other. Such interaction either may involve the template and the "regulator" (inducer or represser) or may involve template, template product, and regulator. In line with current assumptions,[3] this template may be one immediately involved in the production of enzyme protein.

Since action of the regulator—in particular, of the inducer—does not appear to be a *sine qua non* of enzyme formation,[1] the cell is thought to have, prior to contact with the inducer, the essential information required for enzyme structure. Accordingly, the inducer would seem to act not by furnishing a prototype for the configuration of the enzyme molecule but, rather, by improving the performance of the template. Thus the inducer would assist the template catalyst, and may be considered a "promoter." In contrast, a represser may be compared to a catalyst "poison."

It is proposed that inducers and repressers act by affecting the rate of dissociation

of a template product from its template. Repression could then be the result of binding of newly formed enzyme protein to its site of synthesis through the agency of the represser involved. Induction may reflect the neutralization of a binding effect which, in the absence of the inducer, would tend to hold the nascent protein near its template. It is considered that rapid removal of template product from template will accelerate enzyme formation, while occupation of a template by its product will prevent the template from functioning in further enzyme synthesis. It is not necessary to assume that the represser causes binding of enzyme to template by acting as a "bridge"; it is possible that the represser causes a change in macromolecular configuration which results in binding. Similarly, an inducer may act either through the local "neutralization" of a bridge group or through an effect on macromolecule configuration.

Regulators may be expected to show greater or lesser tendencies to depart from their locales of action. Thus, in line with Pollock's observations,[4] the inducer of penicillinase (in contrast to inducers of β-galactosidase) appears to be tightly held at the induction site.

In the present view of regulator action, the separation of template product from template is regarded as a more or less well-defined, single event. However, this view is not necessarily in conflict with a (simultaneous) multiple partial functioning of a template, such as is envisaged by Dalgliesh.[5] In "two-dimensional" protein synthesis,[6] however, one wonders to what extent such partial functioning of "one-dimensional" templates is likely to occur.

The hypothesis here proposed, which will be referred to as the "regulator hypothesis," implies that all enzymes, be they constitutive or adaptive, inducible or repressible, are synthesized in the same general manner but may differ from case to case in susceptibility to regulators. This picture differs from Cohn and Monod's[7] "generalized induction hypothesis" (cf. Vogel[1]).

The Regulator Hypothesis and the "Final Shaping" of Enzymes.—In its general form, the regulator hypothesis is concerned with the dissociation of a template product from its template. Let it now be assumed that the template product is the nascent enzyme protein and that the regulator acts in a region that corresponds to the dynamic site of the "finally shaped"[8] (see below) enzyme molecule. If so, the regulator would be expected to be in close physical contact with the nascent dynamic site at the very moment that the template product separates from the template. Such contact may well be of considerable consequence for the fruitful final shaping of the enzyme molecule, particularly in the folding of the nascent enzyme protein or in the aggregation of template products. A directed folding process in protein biogenesis has been considered by previous authors, especially in connection with antibody formation (Pauling-Haurowitz-Mudd hypothesis; cf. Haurowitz[6]). In principle, the importance of the final shaping process has been anticipated—for example, in the specific-precursor postulates of Monod[9] and of Pollock.[4]

Lipmann[10] has recently drawn attention to the final shaping of proteins as distinct from the emergence of a defined sequential arrangement ("patternization") of component amino acids. While, as suggested above, final shaping may be affected by regulators, this process is considered to be capable of occurring in the absence of regulators. For instance, appropriate folding of nascent enzyme protein could

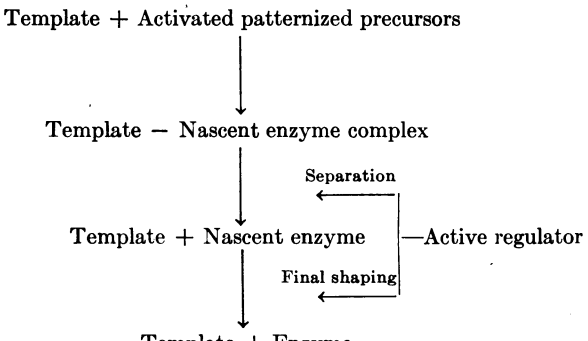

FIG. 1.—Scheme of regulator action

be a spontaneous consequence of the patternization involved.[10] The possible role of regulators in influencing (*a*) the separation of template product from template and (*b*) the final shaping is presented schematically in Figure 1.[11]

Comments on the Regulator Hypothesis.—The regulator hypothesis is generally consistent with and supported by the available data on enzyme induction and enzyme repression. This hypothesis readily accounts for the observation (cf. Pollock[4]) that inducible enzymes usually show a "basal" enzyme level in the absence of added inducers. The basal level would correspond to the rate of enzyme production that the cell is capable of achieving without the benefit of the promoting effect of the inducer.

The emergence of mutants in which an originally inducible enzyme has become constitutive (cf. Monod and Cohn[12]) can be visualized, at least in some cases, as involving a template alteration such that the corresponding template product is enabled to dissociate rapidly enough from its site of formation without aid from a regulator substance; in other words, there would be a change from inducible to indifferent enzyme synthesis.

The present hypothesis is also in harmony with other results, including the finding[13] that there is no necessary relationship between the properties of a given substance as an inducer (or a represser), on the one hand, and a substrate or a complexant, on the other. Again, the regulator hypothesis is in accord with the further findings that different inducers can evoke enzymes of indistinguishable specificity[12] and that an enzyme produced under conditions of repression has been found not to differ detectably from the corresponding nonrepressed enzyme.[1]

A dual regulator function, such as that illustrated in Figure 1, appears to be consistent with available results on induced enzyme formation, including those of Cohn and Torriani.[14] These authors have considered the possibility that the β-galactosidase and the immunochemically related (but enzymatically inactive) Pz protein of *E. coli* are synthesized independently at the same site. In line with this possibility, the increased rate of β-galactosidase formation and the decreased rate of Pz formation observed upon induction may well involve the above-mentioned dual regulator effect. However, since it has not been definitely established that β-galactosidase and Pz actually are produced at the same site, other explanations for the relation between these two proteins remain to be considered.

The Regulator Hypothesis and Other Theories of Enzyme Formation.—In a recent review on theories of enzyme adaptation, Mandlestam[15] has aptly commented on the unusually high ratio of theories to facts in this field; he has also pointed out, however, that it is unlikely that this subject would have advanced so rapidly if the theories had not been formulated. A comparison of the regulator hypothesis with some earlier concepts might therefore not be without some value.

The major theories of induction can be classified as (I) those that depend on some kind of combination of inducer with (finally shaped) enzyme (for either equilibrium displacement or enzyme stabilization or template-like action) and (II) those that do not.

Group I includes Yudkin's[16] mass-action theory and Mandlestam's[17] extended-mass-action theory; Spiegelman's[18] plasmagene theory and Monod's[9] specific-precursor theory; and Campbell and Spiegelman's template-inducer-enzyme complex theory (cited by Spiegelman and Campbell[3]), in which such a triple complex is considered to be the only structure that can effectively and rapidly lead to enzyme synthesis. Group II contains Pollock's[4] organizer hypothesis (which has been extended by Cohn and Monod[7]), Monod and Cohn's[12] inducer-prototype hypothesis, and the present regulator hypothesis.

Arguments against those theories in Group I which invoke a binary complex of (finally shaped) enzyme and inducer have been presented by Monod *et al.*[13] These authors showed that, for β-galactosidase of *E. coli*, inducer function is separable from substrate or complexant functions. Their arguments do not apply to a triple complex, such as is contemplated by Campbell and Spiegelman. The triple-complex hypothesis was advanced primarily to account for "long-term adaptation" in yeast.[3] Since, however, other possible explanations[19] of long-term adaptation have not been excluded, one is not forced to assume that a given finally shaped enzyme participates in the synthesis of its own molecular species. It is also worthy of note that the above-mentioned results of Monod *et al.*[13] do not provide evidence against a complex involving an inducer and the corresponding nascent enzyme (which may be similar to, but not identical with, the finally shaped enzyme in affinity for complexants and in enzymatic activity).

Group II, which includes hypotheses that do not invoke an active participation of finally shaped enzyme in the production of its species, can be subdivided into the organizer and inducer-prototype hypotheses, on the one hand, and the regulator hypothesis, on the other. The inducer-prototype hypothesis assumes that the steric configuration of an enzyme's active site is directly derived from the inducer.[12, 20] Similarly, the organizer hypothesis has been interpreted[7] as implying that the characteristic pattern of an enzyme's dynamic site is imposed by a co-organizer ("active" inducer). Arguments against such a prototype or co-organizer function of the inducer have recently been discussed.[1] In the regulator hypothesis, the assumption of prototype function is avoided.

Summary.—A hypothesis (regulator hypothesis) has been proposed to account for induced and repressed enzyme formation, which are viewed as analogous phenomena in terms of function and of mechanism. Regulators (inducers or repressers) are thought to act, respectively, by promoting or impeding the separation of template product (nascent enzyme protein) from template. An effect of regulators on the final shaping of enzyme molecules is also contemplated. Regulator action is pictured as a control mechanism rather than as a *sine qua non* of enzyme biogenesis.

* This work was aided by a contract between the Office of Naval Research, Department of the Navy, and Yale University, and by the Damon Runyon Memorial Fund.

[1] H. J. Vogel, in W. D. McElroy and B. Glass (eds.), *The Chemical Basis of Heredity* (Baltimore: Johns Hopkins Press, 1957), p. 276. In this paper other results best interpreted as enzyme repres-

sion are cited. A convincing analysis of repressed enzyme formation in the pathway leading to pyrimidine compounds has been carried out by R. A. Yates and A. B. Pardee (*J. Biol. Chem.* [in press] and personal communication).

[2] Induction and repression may respectively be brought about by inducers and repressers acting separately. When, however, an inducer and a represser of one and the same enzyme are present simultaneously, an antagonistic effect involving the two regulators may be expected to result. More generally, it is possible that inducer or represser action is antagonizable by substances that, in themselves, may or may not be regulators.

[3] S. Spiegelman and A. M. Campbell, in D. E. Green (ed.), *Currents in Biochemical Research, 1956* (New York: Interscience Publishers, Inc., 1956), p. 115.

[4] M. R. Pollock, in E. F. Gale and R. Davies (eds.), *Adaptation in Micro-organisms* (Cambridge: At the University Press, 1953), p. 150.

[5] C. E. Dalgliesh, *Science*, **125**, 271, 1957.

[6] F. Haurowitz, *Chemistry and Biology of Proteins* (New York: Academic Press, Inc., 1950).

[7] M. Cohn and J. Monod, in E. F. Gale and R. Davies (eds.), *Adaptation in Micro-organisms* (Cambridge: At the University Press, 1953), p. 132.

[8] Final shaping may include one or more of the following: (*a*) the folding or other intramolecular arrangement of a nascent template product; (*b*) the aggregation of products from the same or from different templates; (*c*) the combination of a template product with another type of substance; (*d*) the breaking of one or more bonds within a template product; and (*e*) the removal of a portion of a template product. The term "dynamic site" refers to that region of an enzyme which is primarily associated with the enzyme's characteristic catalytic action.

[9] J. Monod, *Growth*, **11**, 223, 1947.

[10] F. Lipmann, in D. E. Green (ed.), *Currents in Biochemical Research, 1956* (New York: Interscience Publishers, Inc., 1956), p. 241.

[11] Clearly, regulator action may depend either on both these effects or primarily on one or the other.

[12] J. Monod and M. Cohn, *Advances in Enzymol.*, **13**, 67, 1952.

[13] J. Monod, G. Cohen-Bazire, and M. Cohn, *Biochim. et Biophys. Acta*, **7**, 585, 1951. This paper includes an example of the specific inhibition of induced enzyme formation by an analogue of the inducer. Such inhibition would fall under the definition of enzyme repression given previously (Vogel, *op. cit.*).

[14] M. Cohn and A. Torriani, *Biochim. et Biophys. Acta*, **10**, 280, 1953. See also M. Cohn, Discussion of paper by Cohn and Monod, *op. cit.*, p. 149.

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[19] J. Monod, in O. H. Gaebler (ed.), *Enzymes: Units of Biological Structure and Function* (New York: Academic Press, 1956), p. 7.

[20] The inducer would thus act as the master model or prototype of the (structurally complementary) dynamic site.

THE SITES OF ORTHOPHOSPHATE UPTAKE BY BARLEY ROOTS

BY C. E. HAGEN, J. E. LEGGETT, AND P. C. JACKSON

AGRICULTURAL RESEARCH SERVICE, UNITED STATES DEPARTMENT OF AGRICULTURE, BELTSVILLE, MARYLAND

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Kinetic analysis of measurements of steady-state uptake has established the extent to which the $HPO_4^{=}$ and $H_2PO_4^{-}$ ions contribute to the total uptake of orthophosphate by excised barley roots.[1] The binding compounds involved in the rate-limiting step of orthophosphate uptake are shown here to be components of the