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THE CONTROL OF THE FORMATION OF SPECIFIC PROTEINS IN BACTERIA AND IN ANIMAL CELLS

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In 1953, Monod and Cohen-Bazire as well as Cohn, Cohen, and Monod showed that an amino acid may repress the *last* enzyme in the biochemical pathway which leads to the formation of that amino acid.

Four years later, H. J. Vogel found that arginine, when added to a growing bacterial culture, represses the formation of acetyl ornithinase (one of the *early* enzymes along the biochemical pathway leading to arginine), which converts acetyl-ornithine into ornithine. This observation provided rather strong circumstantial evidence in favor of the view that enzyme repression may be part of the normal regulatory mechanism of enzyme formation in bacteria.

Subsequently, it occurred to Werner Maas that inducers which enhance the formation of an enzyme when added to a growing bacterial culture may perhaps be capable of doing so *only* because there is a repressor present in the cell, and that the inducer might perhaps do no more than inhibit some enzymes which are involved in the formation of the repressor. Thus the inducer would enhance the *formation* of an enzyme only because it reduces the concentration of the repressor in the cell. (Oral communication, April, 1957.)

At that time, the best investigated case of enzyme induction was the induction of the enzyme β-galactosidase. Milton Weiner helped my understanding of the induction of this enzyme greatly by pointing out that its induction must be considered in conjunction with the biochemical pathway leading from galactose to glucose-1-P. (Oral communication, January, 1957.)

The induction of β-galactosidase in a culture of bacteria growing on succinate or lactate, as the carbon source, is inhibited by adding glucose to the growing culture. From this I was led to infer that some metabolite, intermediate in the sequence of metabolites lying between galactose and glucose-1-P, is the precursor of the repressor of β-galactosidase. Further, in line with the above quoted suggestion of Werner Maas, I was led to surmise that certain galactosides may induce β-galactosidase, by inhibiting enzymes which lie on the biochemical pathway between glucose 1-P and the intermediate metabolite that may be the precursor of the repressor of this enzyme.

I believe that some such galactosides may in fact inhibit one of these enzymes and this may explain, in part, why the rate of formation of β-galactosidase rises faster than linearly with the intracellular concentration of the inducing galactoside. However, since I was not able to explain on any similar basis the induction of the degradative enzymes by their substrate, I was led to assume that the inducer must be able to exert an effect which goes beyond inhibiting the formation of a repressor. In particular, I was led to believe that a repressor may reduce the rate of formation of the enzyme by combining with an enzyme molecule which is still attached to its enzyme-forming site, and that it may thereby somehow prevent the attached enzyme molecule from leaving its enzyme-forming site.† According to this view the

repressor combines with a specific site, the controlling site, of the "attached" enzyme molecule, and an inducer may then enhance the formation of the enzyme by competing with the repressor molecule for this site.

I was further led to believe that the repressor of the enzyme β-galactosidase might be a molecule composed of two moieties. One of these (which we may call the metabolite moiety) might be a galactoside and the other (which we may call the *R* moiety) might perhaps be a polynucleotide. Certain galactosides would then enhance the formation of the enzyme β-galactosidase by competing for the controlling site of the attached enzyme molecule with the metabolite moiety of the repressor.

A model which was based on this mechanism of induction and repression of enzyme formation in bacteria was presented by me in a paper given at the annual meeting of the German Chemical Society in Berlin (Oct. 7, 1957) and in lectures, given in the subsequent six months, at various institutions actively interested in the problem of enzyme induction.

The model appeared to be capable of accounting not only for enzyme repression but also for some aspects of antibody formation in mammals. It was not then clear, however, whether it might provide a convincing explanation for the phenomenon of lasting immunity. Such lasting immunity manifests itself in the secondary antibody response which may be elicited in mammals such as the rabbit when it is given an injection of an antigen by which it had been immunized earlier. This secondary response can be elicited even if the second injection of the antigen follows the first injection of the antigen after a very long time interval and thus it involves a "memory" which fades away only very slowly.

In the postwar years the study of inducible enzymes received its impetus chiefly from Jacques Monod's studies of the induction of the enzyme β-galactosidase, at the Institut Pasteur in Paris. The induction of this enzyme turned out to be a very complex phenomenon, however, and until recently it could only be inferred that it involved enzyme repression.

Two years ago, when the model here discussed was first presented, it was supported only by scattered experimental facts. In particular the tenet that the *R* moiety might be a polynucleotide was based on rather tenuous circumstantial evidence.

In the last two years, however, very considerable progress has been made in the study of enzyme induction and enzyme repression. Thus an experiment by Arthur Pardee, Francois Jacob, and Jacques Monod has provided evidence in favor of the view that the phenomenon of enzyme repression may play a major role in the induction of β-galactosidase. Recent experiments performed by Luigi Gorini, at the Medical School of Harvard University, and by George Cohen, Francois Gros, Francois Jacob, Werner Maas, Jacques Monod, and Arthur Pardee, at the Institut Pasteur, support the view that enzyme repression may be the key to the understanding of the phenomenon of enzyme induction in general.

One of these recent experiments shows that a bacterial gene which is responsible for the repression of the enzyme β-galactosidase may exert its effect not by causing the synthesis of an enzyme, but rather by causing the synthesis of a molecule which is not a protein. This remarkable finding is consistent with our notion that this

gene might cause the formation of the R moiety of the repressor of β-galactosidase and that this R moiety might be a polynucleotide.

Apparently, the results of all these newer experiments are, so far, still consistent with the model formulated two years ago, and, in this limited sense, they lend support to the model.

REPRESSION AND INDUCTION OF THE FORMATION OF ENZYMES IN BACTERIA

1. *Enzymes Leading to Glucose-1-P.*—There are numerous repressible enzymes involved in the biochemical pathways which lead from a carbon source to glucose-1-P. In general, the formation of such enzymes is inhibited by glucose. This is quite consistent with the views on enzyme repression here adopted and may be readily understood on the basis of the considerations presented by Neidhardt and Magasanik (1956).

I propose to skip the discussion of this large class of enzymes in favor of discussing two other large classes of enzymes which we may designate as "special anabolic enzymes" and "degradative enzymes."

2. *Special Anabolic Enzymes.*—The enzymes which lie along the biochemical pathways that lead to the formation of an amino acid, a purine, or a pyrimidine, we shall designate as "special anabolic enzymes." We shall single out the enzymes, E_i, involved in the biosynthesis of the amino-acid arginine as being representative for the enzymes of this class.

One of these enzymes, ornithine transcarbamylase, which converts ornithine into citrulline, has been studied by Luigi Gorini and Werner Maas. In a certain strain of coli, the formation of this enzyme may be repressed by adding arginine to a growing bacterial culture. If the intracellular concentration of arginine is lowered in a mutant which cannot convert citrulline into arginine by growing it at a slow rate in a chemostat, with arginine as a controlling growth factor, then the rate of production of the enzyme is raised by a factor of about 25.

We shall refer to enzymes whose rate of production can be thus raised as "boostable" enzymes.

The high rate of enzyme production which may be obtained in the case of such a "boostable" enzyme by lowering the intracellular concentration of a controlling metabolite may represent the "full rate" at which an enzyme-forming site is capable of synthesizing the enzyme.

There are other strains of coli in which the rate of formation of ornithine transcarbamylase cannot be boosted by lowering the intracellular concentration of arginine or any other *known* metabolite. We shall not discuss these "unboostable" strains in the present paper.

We do not assume that the molecule responsible for the repression of ornithine transcarbamylase in the "boostable" strains is arginine itself. We rather assume that the repressor REP_i of the enzymes E_i is a composite molecule which consists of two moieties. One of these, the "metabolite" moiety, is arginine; the other is a moiety which we shall designate by R_i and to which we shall refer as the R-moiety. Thus we may write for the chemical formula on the repressor REP_i

$$REP_i = (\text{arginine} - R_i)$$

As stated above, the R moiety of such a repressor molecule might possibly be a polynucleotide.

Our model for the repression of the enzymes E_i, involved in the biosynthesis of arginine, is as follows:

We assume that the polypeptide chain of the enzyme molecule is synthesized along a specific enzyme-forming site, which determines the amino-acid sequence of the polypeptide. This polypeptide folds up to form the enzyme molecule, but this molecule remains attached, perhaps through a covalent bond, to the enzyme-forming site. If there are repressor molecules present in the cell which are specific for this enzyme, then the metabolite moiety of a repressor molecule may reversibly combine with the controlling site of the attached enzyme molecule (and the *R*-moiety might perhaps reversibly combine with the purine and pyrimidine base of the enzyme-forming site itself). We postulate that an "attached" enzyme molecule, which is so combined with a repressor molecule, cannot leave its enzyme-forming site and accordingly the formation of the enzyme may thus be repressed.

Concerning the mechanism of the action of the repressor we may assume—*pour fixer les idées*—that there is some universal enzyme *U* present in the cell which can split the covalent bond that ties newly-formed enzyme molecules to their enzyme-forming site. The repressor molecule, when it is combined with an attached enzyme molecule, sets up a "steric hindrance" and may thus prevent the universal enzyme *U* from splitting the covalent bond.

We postulate that the enzymes E_i, lying along a biochemical pathway that leads to an amino acid, have two specific combining sites, the "catalytic" site and the "controlling" site.

In the case of an enzyme which catalyzes an early step in the biosynthetic pathway, leading to an amino acid, a purine or a pyrimidine, there need be little chemical resemblance between the substrate of the enzyme and the amino acid, the purine, or the pyrimidine, which lies at the end of the biosynthetic pathway and which may be capable of repressing the formation of the enzyme. The substrate has a specific chemical affinity to the catalytic site of the enzyme, and the end product of the biosynthetic chain, the amino acid, the purine, or the pyrimidine, has a specific affinity to the controlling site. Therefore in the case of these "early" enzymes, the combining specificity of the catalytic site and of the controlling site might be quite different.

In the case of the enzymes which lie towards the end of the biochemical pathway, however, the substrate of the enzyme is likely to be a chemical analogue of the "end product" and we may expect it therefore to have specific chemical affinity, not only for the catalytic site but also for the controlling site of the enzyme.

3. *Degradative Enzymes E_j.*—The enzymes involved in the degradation of tryptophane to β-keto-adipic acid by *Pseudomonas fluorescens* may be taken to be representative for another large class of enzymes, the "degradative enzymes." There are seven enzymes involved in the degradation of tryptophane, and the formation of each of them can be enhanced by adding the substrate of the enzyme to the growing bacterial culture.

There is a vast array of compounds which can be oxidized by bacteria such as *P. fluorescens*. R. Y. Stanier estimated their number at more than 50 and estimated that at least 200 enzymes participate in their degradation. The formation of each of these enzymes may be expected to be enhanced by adding its substrate

to the growing bacterial culture, and we may therefore regard these "degradative enzymes" as "inducible" enzymes.

We postulate that the degradative enzymes E_j also have two specific combining sites, a catalytic site and a controlling site. Since in the case of the degradative enzymes the substrate of the enzyme is an inducer of the enzyme, we assume that the substrate of such a degradative enzyme has a substantial chemical affinity for the controlling site as well as the catalytic site. In the case of the degradative enzymes, E_j, we may therefore expect the two combining sites to be similar in their combining specificity for any given metabolite M.

The metabolite moiety of the repressor of a degradative enzyme might be either the substrate itself or else a metabolite further down the degradative pathway which is still a chemical analogue of the substrate.

If the metabolite moiety of the repressor is the substrate itself, and if the concentration of the repressor rises more slowly with increasing intracellular concentration of the substrate than the concentration of the substrate, then the substrate must be an inducer of the enzyme because it competes with the repressor for the attached enzyme molecule.

The concentration of the repressor would rise more slowly than the concentration of the substrate, for instance, if the limiting factor for the rate of production of the repressor were the rate of production of the R moiety of the repressor.

4. *The Coupling Enzymes C_i or C_j.*—We postulate that there may be present in the bacterial cells a class of enzymes to which we may refer as coupling enzymes, C_i and C_j, which couple a specific R moiety, R_i and R_j, to the metabolite M_i and M_j, and thus form the repressor REP_i and REP_j respectively.

Within the class of the degradative enzymes E^j, the corresponding coupling enzymes C_j might be close to being saturated with respect to the metabolite moiety of the repressor. With increasing intracellular concentration of the substrate, the concentration of the repressor would then rise more slowly than the concentration of the substrate and on this basis one may then expect the substrate to be an inducer of the enzyme.

If a compound $\bar{M}$ is a chemical analogue of the metabolite moiety M of the repressor REP, and if the cell cannot transform it into the metabolite moiety M, then such a compound may reduce the concentration of the repressor by inhibiting the coupling enzyme C and thereby enhance the formation of the enzyme E.

Equations Describing Our Model for Enzyme Repression and Induction.‡—In a bacterial culture, growing at a fixed rate, the total repressor concentration ρ_0, that establishes itself in the stationary state, is proportional to the rate at which the repressor molecules are formed.

We may compute ρ, the concentration of the free repressor molecules, from ρ_0, the total concentration of the repressor molecule J by writing

$$\rho = \rho_0 - z\frac{\rho/K}{1 + \rho/K} \tag{1A}$$

where K designates the equilibrium constant for the dissociation of the repressor molecule from the controlling site of the "unattached" enzyme molecules and z the concentration of the enzyme E in the cell.

In (1A) the first term represents the total concentration of the repressor in the

cell and the second term represents the concentration of those repressor molecules which are combined with the controlling site of an "unattached" enzyme molecule. For the sake of keeping our formulae simple we are disregarding here the fact that the repressor may also combine with the catalytic site of the "unattached" enzyme molecules. Accordingly (1A) must be amplified when this becomes relevant to the issue considered.

We may write (1A) also in the form of (1B)

$$z = \rho_0 \left(1 - \frac{\rho}{\rho_0}\right) + K \left(\frac{\rho_0}{\rho} - 1\right) \tag{1B}$$

We shall designate by τ the average time for which a newly-formed enzyme molecule remains tied to its enzyme-forming site, when it is *not* combined with a repressor molecule at its controlling site. We shall assume that τ is large compared to the time that it takes for the polypeptide to be formed and to fold up to form the attached enzyme molecule. In these circumstances we may say that there is practically always an enzyme molecule attached to the enzyme-forming site, and this enzyme molecule is either combined with a repressor molecule or it is not.

On the basis of our model we may then say that the rate of formation of an enzyme, when it is limited by the presence of a repressor molecule in the cell, is given by

$$\text{rate} = q/\tau \text{ per enzyme-forming site, per unit time} \tag{2}$$

where q is the probability that the attached enzyme molecule is *not* combined at its controlling site with a repressor molecule.

We may write for this probability q, in the presence of a repressor REP and an inducer $\bar{M}$

$$q = \frac{1}{1 + \mu/K_{\bar{M}}^* + \rho/K^*} + \frac{\mu/K_{\bar{M}}^*}{1 + \mu/K_{\bar{M}}^* + \rho/K^*} \tag{3}$$

μ and ρ are the intracellular concentrations of the inducer $\bar{M}$ and the free repressor REP, respectively; $K_{\bar{M}}^*$ and K^* are the equilibrium constants, for the reversible dissociation of the inducer molecule $\bar{M}$ and the repressor molecule REP, respectively, from the controlling site of the attached enzyme molecule.

In (3) the first term gives the fraction of the attached enzyme molecules which are not combined at their controlling site with either a repressor molecule REP or an inducer molecule $\bar{M}$. The second term represents the fraction of the attached enzyme molecules which are combined at the controlling site with an inducer molecule $\bar{M}$.

Formula (3) holds true if the average time that it takes an inducer molecule to dissociate from the controlling site of the attached enzyme molecule is short in comparison with τ.

We may write from (2) and (3) for the rate of enzyme formation per enzyme-forming site per unit time

$$\text{rate} = \frac{1}{\tau} \frac{1 + \mu/K_{\bar{M}}^*}{1 + \mu/K_{\bar{M}}^* + \rho/K^*} \tag{3A}$$

The concentration of an enzyme in a bacterium that grows at a fixed rate is pro-

portional to the rate at which the enzyme is formed. Accordingly, we may write for z, the intracellular concentration of the enzyme

$$z = z^* \frac{1 + \mu/K_{\bar{M}}^*}{1 + \mu/K_{\bar{M}}^* + \rho/K^*} \tag{4}$$

where z^* is the concentration which the enzyme might attain in the cell, in the absence of any repressor.

In the absence of the inducer $\bar{M}$ we may write

$$z = z^* \frac{1}{1 + \rho/K^*}. \tag{5}$$

Equations (4) and (5) give the concentration of the enzyme in the cell independent of how many enzyme-forming sites are present in the cell which synthesize the same enzyme. In (4) and (5), z^* represents the enzyme concentration which is obtained from these formulae when ρ, the repressor concentration, becomes zero.

It should be noted, however, that when the repressor concentration becomes very small and the concentration of the enzyme becomes correspondingly large, the repressor concentration may cease to be the limiting factor for the rate of enzyme production and something else may become rate-limiting. For this reason the enzyme concentration in the cell might not actually reach the value z^*, if the repressor concentration goes to zero.

We may now introduce into our formulae the repression factor λ which is defined by

$$\lambda = \rho_0/K^*.$$

We may then write (5) in the form

$$z = z^* \frac{1}{1 + \lambda\rho/\rho_0} \tag{5A}$$

and this we may also write in the form

$$\rho = \frac{\rho_0}{\lambda} \frac{1 - z/z^*}{z/z^*} \tag{5B}$$

We shall throughout the rest of our discussion invariably assume that we have

$$z^* \gg \rho_0. \tag{6}$$

The Simplified Equations.—For the enzymes E for which we may write

$$K \gg z^*$$

the second term in (1A) can be neglected and we may then write

$$\rho = \rho_0.$$

In this case (4) and (5) may be written in the form of

$$z = z^* \frac{1 + \mu/K_{\bar{M}}^*}{1 + \mu/K_{\bar{M}}^* + \rho_0/K^*} \tag{7}$$

and

$$z = z^* \frac{1}{1 + \rho_0/K^*} = \frac{z^*}{\lambda + 1} \tag{8}$$

When these simplified equations hold, then the rate of enzyme formation is independent of the concentration of the enzyme in the bacterium. Accordingly, if an inducer is added to a growing bacterial culture at a given point in time, the rate of enzyme formation will rise to a new value at the time when the inducer is added and from then on it will remain constant. This could be verified by studying the kinetics of the induction of the enzyme.

It may be seen from (7) that if the presence of the inducer $\bar{M}$ does not affect ρ_0, the concentration of the repressor molecules in the cell, then the rate of formation of the enzyme cannot rise any faster than linearly with the intracellular concentration of the inducer.

If it is found that the enzyme concentration rises faster than linearly with the intracellular concentration of the inducer, we may then expect either that the inducer inhibits an enzyme involved in the formation of the metabolite moiety of the repressor or that it inhibits the enzyme C which couples the metabolite moiety of the repressor to the R moiety of the repressor, or that it does both.

Predictions Based on the "Simplified" Equations.—On the basis of the model as described by the above given simplified equations we may expect the following:

(a) If the cells of a bacterial strain are incapable of converting $\bar{M}$ (a chemical analogue of the repressor's metabolite moiety) into M (the repressor's metabolite moiety itself), and if the concentration of M is fixed, then the chemical analogue $\bar{M}$ may enhance the formation of the enzyme, provided that it can get into the bacterial cell.

The chemical analogue $\bar{M}$ may be an inducer of the enzyme in such a bacterial strain, either because it competes with the metabolite moiety of the repressor for the controlling site of the enzyme molecule which is attached to its enzyme-forming site, or because it competes with the metabolite M for the coupling enzyme C which joins the metabolite moiety to the R moiety of the repressor, or for both of these reasons.

In the case of the bio-synthetic pathway leading to arginine the substrate of a *late* enzyme is a chemical analogue of arginine. Accordingly, we may expect such a substrate to induce the enzyme in mutant bacterial strains which cannot convert the substrate into arginine. Thus we may expect the enzyme ornithine transcarbamylase, which converts ornithine into citrulline, to be inducible by citrulline, in a mutant strain which cannot convert citrulline into arginine.

Luigi Gorini has observed that ornithine induces the enzyme ornithine transcarbamylase in such a mutant, if the intracellular concentration of arginine is kept *moderate* by growing the bacterium *at a fast rate* in a chemostat with arginine as a controlling growth factor. (Oral communications, 1959.) (If the intracellular concentration of arginine were kept low by growing the bacterium at a slow rate in the chemostat, then the enzyme level would be boosted to a high value and the inducing effect of ornithine would not be observable.) Since this mutant converts ornithine into citrulline, Gorini's observation is consistent with the views here presented.

(b) There might exist mutants in which the R moiety of the repressor is produced, but it is produced at such a low rate that its production may be the limiting factor for the production of the repressor when the intracellular concentration of the metabolite moiety of the repressor is not too low. On the basis of (7), we may then say that in such a mutant the formation of the enzyme should be enhanced by adding the repressor's metabolite moiety to the growing bacterial culture. Accordingly, for such a mutant the metabolite moiety of the repressor may be an inducer of the enzyme.

Thus, in such a mutant, arginine (for instance) should be an inducer of the enzyme ornithine transcarbamylase and it should be an inducer of this enzyme for one reason only, i.e. because it may compete with the repressor for the controlling site of the attached enzyme molecule.

(c) There may exist mutants which are not capable of producing the R moiety of the repressor. In such a mutant the enzyme may be produced at the full rate and accordingly the enzyme level would be very high. In such a mutant it should not be possible to repress the enzyme by adding the metabolite moiety of the repressor to the growing bacterial culture. Mutants of this type may be designated as "absolute constitutive strains."

Both Luigi Gorini and Werner Maas have obtained from a strain of coli, in which the enzyme ornithine transcarbamylase is repressible by arginine, mutants in which the enzyme is always maintained at a high level and is not repressible by arginine. (Oral communications, 1959.) Conceivably these might be mutants in which the R moiety of the repressor is not formed, i.e., they might be absolute constitutive strains.

The Case of $z^* \gg K$. — We may postulate here, for the sake of argument, a set of constants for "wild type" bacteria which might be as follows:

$$z^* = 10^{-4} \text{ mol/l}, \qquad \rho_0 = 10^{-6} \text{ mol/l};$$
$$K = 10^{-5} \text{ mol/l}; \qquad K^* = 10^{-10} \text{ mol/l}$$

The postulated value of $z^* = 10^{-4}$ mol/l would mean that a fully boosted enzyme amounts to about 10 per cent of the cell proteins.

The value of $\rho_0 = 10^{-6}$ mol/l postulated would be consistent with the assumption that there may be one thousand different repressors present in a bacterium and the assumption that the R moiety of these repressors is a polyribonucleotide of a molecular weight of about 2,000, without having to assume a larger amount of soluble *RNA* in the bacterium than is usually found in bacteria.

The postulated value of $K = 10^{-5}$ mol/l means that the free energy change for the combination of the repressor with the controlling site of an unattached enzyme molecule is about $\Delta F = 7{,}000$ cal/mol. This appears to be a reasonable value if we assume that only the M moiety of the repressor has a specific chemical affinity to the controlling site of the unattached enzyme molecule.

The postulated value of $K^* = 10^{-10}$ mol/l corresponds to a free energy change of $\Delta F = 14{,}000$ cal/mol for the combination of the repressor with the enzyme molecule that is attached to its enzyme-forming site. This appears to be a reasonable value if we assume that the M moiety of the repressor has a specific chemical affinity to the controlling site of the enzyme molecule and that the R moiety of the repressor

has a specific chemical affinity to the purine and pyrimidine bases of the enzyme-forming site itself.

For the above postulated set of constants we have

$$z^* \gg K$$

and accordingly the simplified equations do not strictly speaking hold.

Nevertheless, for this set of constants, ρ, the free repressor concentration, would be very close to ρ_0, because the enzyme is strongly repressed. We may write for the repression factor λ, for our set of constants,

$$\lambda = \rho_0/K^* = 10^4$$

and we have accordingly for the above postulated set of values

$$\frac{z^*}{K(\lambda + 1)} = 10^{-3} \ll 1.$$

In these circumstances ρ may be close to ρ_0.

Limiting ourselves, for the moment, to a set of constants where we have

$$K \gg \rho_0$$

we may now say the following:

If we have to deal with a *mutant* in which the repressor is produced at a very low rate, so that the repression factor λ is small, and if we have

$$\frac{z^*}{K\ (\lambda + 1)} > 1,$$

then ρ, the free repressor concentration, may be much smaller than ρ_0 and, accordingly, the enzyme level in the cell may then be rather high. This may be seen from (1A) as follows:

We may write for the second term in (1A)

$$z \frac{\rho/K}{1 + \rho/K} > \frac{z^*}{\lambda + 1} \frac{\rho/K}{1 + \rho/K} \approx \frac{z^*}{\lambda + 1} \frac{\rho}{K}$$

and thus we obtain from (1A)

$$\frac{\rho_0}{\rho} > 1 + \frac{z^*}{K(\lambda + 1)}$$

and from (5A) we obtain

$$z > \frac{1}{1 + \lambda/\{1 + z^*/[K(\lambda + 1)]\}} z^*.$$

Accordingly, strains of this type may maintain an enzyme level which may be high and which might come close to the fully boosted enzyme level z^* of an "absolute constitutive mutant."

Paraconstitutive Enzymes.—If for an enzyme we have

$$\rho_0 \gg K$$

and if the other constants fall within certain ranges, then the concentration of such

an enzyme may be maintained in the cell either at a low stable value or at a high stable value—in the absence of any inducer.

An enzyme for which this holds we shall designate as "paraconstitutive."

The concentration of such a paraconstitutive enzyme may be maintained indefinitely at a low level in a proliferating cell culture. But, if once the enzyme concentration is raised to a sufficiently high level and maintained there long enough to establish a stationary state, from there on a high rate of production of the enzyme may be permanently sustained—even in the absence of an external, or internal, inducer. Further, when such a cell, which sustains a high enzyme level, divides, then the daughter cells which it generates will also sustain a high enzyme level. Thus the property of sustaining a high enzyme level is hereditary even though the inheritance is not genic.

It is not possible to say whether paraconstitutive enzymes exist in bacteria. If they did exist, it would be somewhat difficult to recognize them, because if they have arisen in the normal course of evolution then it is likely that by now the enzyme would be sustained at a high level, and the paraconstitutive mutant would thus give the appearance of a constitutive mutant.

It might well be, however, that the condition $\rho_0 \gg K$ is not fulfilled for any of the bacterial enzymes. Perhaps, in order to have this inequality hold for an enzyme, it is not sufficient for the controlling site of the enzyme to have a specific combining affinity for the metabolite moiety of the repressor, but it is also necessary for it to have a specific combining affinity for one or more of the units which constitute the R moiety of the repressor.

Paraconstitutive Enzymes and Cellular Differentiation.—Even though the inherent instability of the enzyme-forming system which is described by our equations might play no role in bacteria, it may still be of great interest. It is conceivable that it might play a major role in certain types of cellular differentiation, in higher organisms such as mammals.

There is no need to assume (and in the case of amphibia there may indeed be reason to doubt) that the early cellular differentiation involved in the formation of ectoderm, mesoderm, and endoderm, is of this type. Still, cellular differentiation in the later phases of embryonal development, where an organizer is involved, might conceivably be based on the inherent instability of the enzyme-forming system here discussed. It is not possible, however, to substantiate this at present on the basis of available experimental evidence, and it may be very difficult to substantiate it even through experiments devised for the purpose.

Experimental confirmation of the view that the model here discussed may provide the molecular basis of a certain type of cellular differentiation in mammals might, however, be obtained in the case of antibody formation in mammals or birds. An antibody need not have any enzymatic activity, but we shall assume that it is in some way related to certain degradative enzymes E_j, present in the mammalian cell, and that the rate of formation of an antibody A_j is under the control of the repressor which is specific for the related enzyme E_j.

Whether an enzyme makes its appearance in cellular differentiation, provoked by an organizer, or whether a specific antibody is formed in response to the injection of an antigen, in either case we may assume that the event is triggered by a transient reduction of the concentration of a specific repressor.

If antibodies are paraconstitutive proteins, then, once the concentration of a particular antibody is raised to a sufficiently high level in a lymphatic cell, that cell and all its daughter cells may maintain a high rate of production of that antibody. This would then represent the kind of memory which may form the basis of the so-called secondary response.

The Enzyme-Forming System of the Mammalian Cell.—We assume that the enzyme-forming system in the mammalian cell is described by the same equations which we have postulated for the bacteria. There are certain differences, however, between the mammalian cells here discussed and the bacterial cells. Under physiological conditions bacterial cells as a rule proliferate. Accordingly, in bacteria there is no need for protein turnover, nor do we have any reason to assume that the R moieties of the repressors are hydrolyzed in bacteria at an appreciable rate. In these circumstances, in bacterial cells ρ_0, the total concentration of a particular repressor in the cell, and z^*, the concentration attained by an enzyme which is produced at the full rate, are both determined by the rate of two processes, i.e., the rate at which these entities are formed and the rate at which these entities are diluted through the growth of the bacteria.

In the case of mammalian cells which are not in a state of rapid proliferation, we may in general assume that ρ_0, the concentration of the repressor, and z^*, the concentration of an enzyme which is produced at the full rate, are also determined by the rate of two processes, i.e. the rate at which these entities are produced and the rate at which they are hydrolyzed.

The rate of hydrolysis of the proteins is presumably determined by the level of activity of the proteolytic enzymes in the cell. The rate of hydrolysis of the repressors is presumably determined by the level of activity of some hydrolytic enzyme H which may universally hydrolyze the R moieties of all of the repressors.

We shall assume that the R moiety of the various specific repressors is hydrolyzed in the cell at the same rate whether the specific repressor is free or is combined with the controlling site of an "unattached" enzyme molecule. This is not an unreasonable assumption because the repressor molecule combines with an unattached enzyme molecule in large part by virtue of the chemical affinity of the repressor's M moiety to the controlling site of the enzyme molecule. If the R moiety is indeed a polynucleotide, then the first, or the first few, bases of the polynucleotide may also combine with the controlling site of an unattached enzyme molecule, but the rest of the nucleotide will presumably remain freely exposed to the hydrolytic action of our hypothetical enzyme H.

We shall presently show that if the relevant constants fall within a certain range, quoted below, then according to equations (1) and (5) the enzyme concentration z exhibits the characteristics which we have attributed to paraconstitutive enzymes.

If we equate z given by (1B) and given by (5A), we obtain a cubic equation for ρ. For the below-quoted range of the constants this cubic equation has three positive roots which represent the self-sustaining free repressor concentrations. Accordingly, there may be three self-sustaining pairs of values for ρ and z which we shall designate (in the order of increasing values of z and decreasing values of ρ) with ρ_1, z_1, ρ_2, z_2, and ρ_3, z_3.

Of these, three self-sustaining pairs of values the first and the last pair, ρ_1, z_1 and

ρ_3, z_3 represent stable points. The intermediate pair ρ_2, z_2 represents an unstable point.

We shall refer to z_3 as a high stable enzyme concentration and to ρ_1 as the high stable repressor concentration. To ρ_2 we shall refer as the "critical" repressor concentration.

In the case of a paraconstitutive enzyme, if ρ, the free repressor concentration, is lowered below ρ_2, the critical free repressor concentration (for a period of time which is sufficiently long for the establishment of a stationary state), and if thereafter the free repressor concentration is permitted to find its own level, then the free repressor concentration will decrease to its low stable value ρ_3. Correspondingly, the enzyme concentration will rise to its high stable value z_3.

For any arbitrarily chosen value of ρ which we maintain in the cell long enough to establish a stationary state, we can determine from equations (1B) and (5A) whether the free repressor concentration would thereafter fall or rise in the cell when it is allowed to find its own level. In order to make this determination we substitute the arbitrarily chosen value ρ (to which we shall refer as the test value) into (1B) and (5A), and find from both of these expressions the corresponding value for z.

The rate of enzyme production, for a given free repressor concentration, is expressed by (5A) and if the value for z obtained from this expression is higher than the value for z obtained from (1B), then we may say that the repressor concentration will decrease when it is permitted to find its own level. In the opposite case we may say that the repressor concentration will increase when it is permitted to find its own level.

We propose to utilize below this simple rule, in order to establish the fact that there exist two stable self-sustaining values ρ_1 and ρ_3, if the constants fall within the range quoted below.

We shall now show that if we have

$$\frac{z^*}{K(\lambda - 1)} > 3 \tag{9}$$

and

$$\rho_0/z^* < 10 \tag{10}$$

then there is a stable point at which we have for the low self-sustaining free repressor concentration ρ_3

$$0 < \rho_3 < \rho_0/\lambda.$$

In order to show this we choose for our test concentration $\rho = \rho_0/\lambda$. Substituting this value of ρ into (5B) we obtain

$$\frac{z(\text{from 5B})}{z^*} = \frac{1}{2}$$

Substituting the same value for ρ into (1A) we obtain

$$\frac{z(\text{from 1A})}{z^*} = \frac{\rho_0}{\lambda}(1 + \lambda) + \frac{K(\lambda - 1)}{z^*}.$$

If we take (9) and (10) into account we see that the value for z (from 1A) is less than the value for z (from 5B). Therefore, ρ, when it is permitted to find its own level (after being held at $\rho = \rho_0/\lambda$ for an adequate period of time), will decrease and move to its low stable value, ρ_3.

For the corresponding high stable value of the enzyme concentration z_3 we may write

$$z^*/2 < z_3 < z^*.$$

Next, we propose to determine ρ_1, the high stable concentration of the free repressor, and ρ_2, the critical concentration of the free repressor. We obtain these self-sustaining values from (1B) and (5A) by writing

$$z \text{ (from 1B)} = z \text{ (from 5A)} \tag{11}$$

and by finding the roots of this equation.

Writing out (11) explicitly we obtain

$$\rho_0\left(1 - \frac{\rho}{\rho_0}\right) + K\left(\frac{\rho_0}{\rho} - 1\right) = z^* \frac{1}{1 + \lambda\rho/\rho_0} \tag{11A}$$

which we may also write in the form

$$\lambda = \frac{1}{\rho_0/z^*(1 - \rho/\rho_0)\rho/\rho_0 + K/z^* - K/z^*(\rho/\rho_0)} - \frac{1}{\rho/\rho_0}. \tag{11B}$$

If we have

$$\frac{\rho}{\rho_0}\frac{\rho_0}{z^*} \gg \frac{K}{z^*} \tag{12}$$

and

$$\frac{z^*}{\rho_0}\frac{1}{1 - \rho/\rho_0} \gg 1, \tag{13}$$

then we may simplify (11B) by leaving off the second term and we thus obtain

$$\lambda = \frac{1}{\rho_0/z^*(1 - \rho/\rho_0)\rho/\rho_0 + K/z^* - K/z^*(\rho/\rho_0)} \tag{14}$$

or

$$(\rho/\rho_0)^2 - (1 - K/\rho_0)\rho/\rho_0 + z^*/\rho_0\lambda - K/\rho_0 = 0 \tag{14A}$$

or

$$\frac{\rho_1}{\rho_0} = \frac{1 - K/\rho_0 + \left|\sqrt{[1 - K/\rho_0]^2 - 4[z^*/\rho_0\lambda - K/\rho_0]}\right|}{2} \tag{14B}$$

and

$$\frac{\rho_2}{\rho_0} = \frac{1 - K/\rho_0 - \left|\sqrt{(1 - K/\rho_0)^2 - 4[z^*/\rho_0\lambda - K/\rho_0]}\right|}{2}. \tag{14C}$$

The quantity under the square root in (14B) and (14C) is positive if we have

$$(1 - K/\rho_0)^2 > {}^4/_5 \tag{15}$$

and

$$\lambda > 5z^*/\rho_0. \tag{16}$$

We shall now, for the purposes of our discussion, assume a set of constants which satisfy the inequalities that we have assumed above and actually compute the ρ_1/ρ_0 and ρ_2/ρ_0 from (14B) and (14C) in order to obtain the values for ρ_1 and ρ_2.

For the purpose of our discussion we assume the following set of constants:

$$z^* = 10^{-3} \text{ mol/l}; \ \rho_0 = 10^{-8} \text{ mol/l}; \ K = 10^{-10} \text{ mol/l};$$
$$K^* = 10^{-14} \text{ mol/l}.$$

This set of values satisfies the inequalities assumed above (which represent a sufficient condition for an enzyme to be paraconstitutive) and, in particular, we obtain for this set of constants $\lambda = \rho_0/K^* = 10^6$ and $z^*/K(\lambda - 1) \approx 10$.

The following comment may be made concerning the particular values chosen for our constants:

$z^* = 10^{-3}$ mol/l means that if, even at zero repressor concentrations, the enzyme were formed at the rate corresponding to (5A), then the amount of enzyme in the cell would just about equal the total protein content of the cell.

$K = 10^{-10}$ mol/l corresponds to a free energy change of $\Delta F = 14{,}000$ cal/mol for the combination of a repressor molecule with the controlling site of an "unattached" enzyme molecule. It seems likely that both the metabolite moiety and the R moiety of the repressor would need to have a substantial chemical affinity to the controlling site of the unattached enzyme molecule in order to have a free energy change of this magnitude.

For the above quoted values of the constants we obtain from (14B) for the high stable repressor concentration $\rho_1 \approx 0.9\ \rho_0$ and from (14C) for the critical repressor concentration $\rho_2 \approx 0.11\ \rho_0$.

This means that it would be sufficient to lower the free repressor concentration to about one tenth of ρ_0 in order to trigger the enzyme-forming system and cause the cell thereafter to maintain, indefinitely, the enzyme at a high concentration, i.e. at a concentration lying somewhere between $z^*/2$ and z^*.

The set of values which we have assumed for our constants was selected because it appears conceivable that a quite similar set of values might hold for antibodies formed in the lymphatic cells of the adult rabbit that are capable of forming antibodies which are specific for an antigen injected into the rabbit.

These cells might be characterized—in comparison to the lymphatic cells of the newborn rabbit and the non-lymphatic somatic cells of the adult rabbit—by an increased level of activity of the hypothetical hydrolytic enzyme H. On this basis, we may assume that the value of ρ_0 and of the repression factor λ that holds for the lymphatic cells of the adult rabbit which are capable of forming antibodies is perhaps ten times lower than the corresponding values in those other cells, which are not capable of forming antibodies.

This possibility is discussed in detail in the following paper, "The molecular basis of antibody formation," which attempts to explain a number of phenomena in-

volved in antibody formation on the basis of the phenomenon of enzyme repression in bacteria.

It is conceivable that in attempting to build a theory of antibody formation on this foundation we may be building a house of cards, for in spite of the rapid progress made in the last two years with respect to enzyme repression in bacteria, many of the conclusions drawn from the experiments are still largely based on circumstantial evidence. This holds in particular for the conclusion, here adopted, that the repressor controls the rate at which the enzyme is formed by the enzyme-forming site rather than the rate of formation of the enzyme-forming site itself.

Clearly, we cannot attempt at present to say how antibodies are actually formed; at best we may be able to say how antibodies might conceivably be formed. But to be able to say even this much might be of some value.

I had the privilege of discussing the thoughts expressed in this paper with Dr. Maurice S. Fox, The Rockefeller Institute, New York, and it is a pleasure to acknowledge his help in clarifying the issues involved.

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† A similar model was proposed independently by O. Maaløe and presented at an informal seminar at the Cavendish Laboratory in Cambridge in 1958; it will be described in *Microbial Genetics* (Cambridge University Press, 1960).

‡ Notations:

REP	is the specific repressor which controls the rate of formation of an enzyme and which is composed of two moieties, an M moiety and an R moiety.
M	is a metabolite, which forms the metabolite moiety of the repressor.
R	is the R moiety of a repressor, which is specific for enzymes lying along a certain stretch of a given biochemical pathway.
K^*	is the equilibrium constant for the dissociation of a repressor molecule from the controlling site of the corresponding enzyme molecule that is attached to its enzyme-forming site.
K	is the equilibrium constant for the dissociation of a repressor molecule from the controlling site of the "unattached" enzyme molecule, present in the cell.
$K^*_{\bar{M}}$	is the equilibrium constant for the dissociation of a chemical analogue $\bar{M}$, of the metabolite M, from the controlling site of an enzyme molecule attached to its enzyme-forming site.
$K_{\bar{M}}$	is the equilibrium constant for the dissociation of the chemical analogue $\bar{M}$ from the controlling site of an unattached enzyme molecule contained in the cell.
ρ_0	is the concentration of the molecules of the repressor REP in the cell.
ρ	is the concentration of the free repressor molecules in the cell, i.e. of those repressor molecules which are not combined with an unattached enzyme molecule.
μ	is a concentration of the chemical metabolite $\bar{M}$ in the cell.
z	is the concentration of the "unattached" enzyme molecules in the cell.
z^*	is the concentration that the enzyme would attain in the cell, for $\rho = 0$, if we disregard the fact that factors, which our formulae do not take into account, may limit the rate of the formation of the enzyme for high values of z.
λ	is the repression factor defined by $\lambda = \rho_0/k^*$.