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*THE MOLECULAR BASIS OF ANTIBODY FORMATION**

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In a preceding paper we have discussed the phenomenon of enzyme repression in bacteria and presented a model for a kind of cellular differentiation which might conceivably play a role in embryonic development. In the present paper we propose to discuss the molecular basis of antibody formation in the light of this model.

The Experimental Facts.—We shall list in the following the major immunological phenomena that we may regard as well established. Throughout this paper we shall restrict our discussion to one animal, the rabbit, and to one class of antigens, soluble proteins.

(1) When a soluble protein antigen is injected into an adult rabbit for the first time, the rabbit responds after about 5 days with the formation of antibodies which are specific for this antigen. Any remaining antigen will be rapidly eliminated from the circulation, and for a while there will be a substantial concentration of antibody present in the circulation. Subsequently the concentration of the antibody in the circulation will gradually decrease and after a while it may become no longer detectable. This is the so-called primary response.

(2) If, a few weeks after we have injected into the rabbit a soluble antigen, such as a foreign serum protein, we inject the same antigen again, then three days later the rabbit may respond with a copious production of antibody and the antigen will be rapidly eliminated from the circulation. The concentration of the antibody in the circulation may reach a high level, then fall off rather slowly and remain appreciable for a long time. This is the secondary response.

In response to the same amount of antigen injected, a much larger amount of antibody may be produced in the secondary response than in the primary response.

The secondary response can be elicited even a very long time after the antibody has disappeared from the circulation, subsequent to the first injection of the antigen. The readiness of the rabbit to exhibit such a secondary response represents some sort of a memory which fades away only very slowly.

(3) Albert Coons has found in the rabbit that if one evokes the secondary response in the manner described above, then, after 48 hours, clusters of cells which contain antibodies specific for the antigen may be found in the lymph nodes which are involved. The clusters found 4 days or 8 days after the injection are on the average larger than the clusters found after 48 hours. The cells which compose these clusters are small and round and have the appearance of plasma cells. Mitotic figures can be seen in the clusters, indicating that there is proliferation of the cells producing the antibody. The clusters vary in size; the larger ones consist of about a hundred or perhaps a few hundred cells.

(4) For a few days after birth, the rabbit is not capable of forming antibody in response to the injection of an antigen. If a newborn rabbit is injected with a *large* quantity of a soluble protein antigen, then later on when this rabbit becomes an adult and is capable of forming antibodies in general, it will still remain incapable of forming antibody against the antigen which was administered to it immedi-

ately after birth, even though by that time the concentration of the antigen in the circulation may have fallen to a very low level. This is the phenomenon of enduring immune tolerance.

(5) If an adult rabbit is given an X-ray dose of about 400 r to its whole body and a few days later an antigen is injected into the rabbit, then the rabbit will not form antibodies against this antigen. After a while the effect of the X-ray exposure will wear off and the rabbit is then again capable of forming antibodies.

If after the X-ray exposure, at the time when the rabbit is not capable of responding with the formation of antibody, a *large* quantity of an antigen, such as a foreign serum protein, is injected into the rabbit, then the rabbit will remain incapable of forming antibodies against this particular antigen, even after the effect of the X-ray exposure has worn off and the concentration of the antigen in the circulation has fallen to a level which is no longer detectable. Thus the X-rayed adult rabbit exhibits the phenomenon of enduring immune tolerance.

(6) If the antigen is injected into the rabbit prior to the exposure to the X-ray dose, then this exposure will not prevent the rabbit from forming antibodies to the antigen.

(7) If a rabbit has been pre-immunized with an antigen, then exposure of the rabbit to an X-ray dose of 400 r a few days prior to the injection of the antigen will not block the secondary response, i.e. in such a rabbit the injection of the antigen will evoke the secondary response even though the injection is given a few days following the administration of the X-ray dose.

The Enzymes of the Lymphatic Cells.—We postulate that, in general, the somatic cells of the rabbit contain a number of diverse biochemical pathways, j, $j + 1$ $j + 2$, etc., and that a number of enzymes lie along each such pathway. This might be represented symbolically by writing

$$M \begin{cases} M_j^1 \xrightarrow{E_j^1} M_j^2 \xrightarrow{E_j^2} M_j^3 \xrightarrow{E_j^3} \cdots\cdots M_j^k \xrightarrow{E_j^k} M_j^{k+1} \\ M_{j+1}^1 \xrightarrow{E_{j+1}^1} M_{j+1}^2 \xrightarrow{E_{j+1}^2} M_{j+1}^3 \xrightarrow{E_{j+1}^3} \cdots\cdots M_{j+1}^k \xrightarrow{E_{j+1}^k} M_{j+1}^{k+1} \\ M_{j+2}^1 \xrightarrow{E_{j+2}^1} M_{j+2}^2 \xrightarrow{E_{j+2}^2} M_{j+2}^3 \xrightarrow{E_{j+2}^3} \cdots\cdots M_{j+2}^k \xrightarrow{E_{j+2}^k} M_{j+2}^{k+1} \end{cases}$$

As this scheme indicates, the metabolites M_j; M_{j+1}; M_{j+2}; etc. of several of these stray biochemical pathways might have a common precursor M.

Let us now consider one of these enzymes, for instance E_j^k. We assume that this enzyme is under the control of the repressor

$$REP_j^k = \{M_j^k - R_j\}$$

We further assume that there is a coupling enzyme C_j^k present in the cell which joins the R moiety of the repressor, R_j, to the M moiety of the repressor, M_j^k. Symbolically this might be indicated by writing

$$M_j^k + R_j \xrightarrow{C_j^k} \{M_j^k - R_j\}.$$

We shall assume in our discussion that the coupling enzymes C are not under the control of any of the repressors here considered.

We shall further assume that both the catalytic site and the controlling site of all these degradative enzymes, E_j, can specifically combine with M_j, the substrate of the enzyme. (See preceding paper.)

The Genes G and the Antibodies A.*—We shall designate by G_j the genes which determine the identity of these degradative enzymes E_j, and we postulate that for each enzyme E_j there is only one corresponding gene G_j contained in each of the two haploid sets of chromosomes. The total number of genes of this category might perhaps be of the order of 10,000 per haploid chromosomal set.

One might be tempted at this point to assume that the enzymes E are themselves the antibodies. We prefer, however, to postulate the following: the genes G have a tendency to get doubled and in the course of evolution each gene G_j may have doubled many times. We assume that when a gene G undergoes doubling, the genes G^* which are formed will in general lack a part, presumably a small part, of the gene G. We assume in particular that the genes G^* differ from the genes G in two respects, which are as follows:

(a) the genes G^* lack the tendency of the genes G to undergo doubling;

(b) the proteins which are determined by the genes G_j^* resemble closely the enzyme E_j which is determined by the corresponding gene G_j but they may lack the catalytic activity of the enzyme. *We postulate that these proteins are the antibodies* A_j.

The controlling site of an antibody A_j has the same specific combining affinity to the metabolite M_j as has the controlling site of the enzyme E_j. Also the catalytic site of an antibody A_j may have the same specific combining capacity for the metabolite M_j as the catalytic site of enzyme E_j, to which it is related, *and if this is the case then we are dealing with a divalent antibody.*

The spontaneously occurring doubling of the genes G might be balanced by spontaneously occurring deletions of the genes G^*, and an equilibrium might be maintained on this basis, in the absence of genetic deaths. In such an equilibrium there might be present a number of genes G^* corresponding to each gene G. However, deletions of the genes G and their mutations to incompetence *would* have to be balanced by the occurrence of genetic deaths, in a state of mutational equilibrium.

There is a limit to the amount of genetic deaths which we may assume to occur per generation, because no species of mammals could remain in existence if the amount of genetic deaths were too high. This consideration does not permit us, however, to set an upper limit for the number of genes G^* present per gene G, because we are assuming that with respect to the genes G^* mutational equilibrium is maintained in the absence of genetic deaths.

We may set an upper limit for the total number of genes G^* on the basis of the amount of *DNA* present in the cell, but this comes out to be very high; *if we assume that the weight of the mammalian gene is about the same as the weight of the bacterial gene, then the amount of DNA in the mammalian cell would be sufficient to account for one million genes.*

The Nature of the Antigen.—For the purpose of our discussion an antigen P_j may be represented as a molecule which is composed of a non-antigenic protein molecule P_0 to which are coupled m identical groups $\bar{M}_j$ (to which we shall refer as a hapten). Accordingly, we may represent such an antigen P_j symbolically, by writing

$$P_j = \{P_0 - (\bar{M}_j)_m\}$$

One can prepare rather simple artificial antigens of this type by diazo-coupling

a hapten, some small molecule, arsanylic acid for instance, to a protein which is not antigenic in the rabbit. If m, the average number of such haptens per protein molecule, is made large enough ($m \geq 10$) we may have a good antigen which will elicit the formation of antibodies specific for the hapten.

If the hapten $\bar{M}_j$ is a chemical analogue of the metabolite M_j, then the antibody molecule A_j is capable of combining with the antigen molecule P_j by virtue of the specific chemical affinity of the catalytic site and of the controlling site of the antibody molecule A_j to the hapten $\bar{M}_j$.

In the case of a natural protein antigen, such as a foreign serum protein, we have a more complicated situation because one protein molecule might carry a certain number, m_1, of one kind of determinant group and also a certain number, m_2, of another kind of determinant group, etc. To these determinant groups we shall, for the sake of brevity, also refer as haptens.

More complex artificial antigens may be prepared by coupling a hapten to a natural protein which is itself antigenic in the rabbit.

The Rabbit Antibody-Antigen System.—If antibody which is obtained from the rabbit is mixed with the antigen for which it is specific, then within certain concentration limits a precipitate may be formed in which each antigen molecule may be combined with a number of antibody molecules, and each antibody molecule may be combined with two antigen molecules. The concentration limits within which such a precipitate is formed define the so-called equivalence zone where the supernatant over the precipitate contains only small quantities of both the antigen and the antibody. The free antigen concentration is exceedingly low in equilibrium with such a precipitate.

A precipitate may also be formed if the rabbit antibody is present in large excess. Such a precipitate may be represented as consisting of units in which a number of antibody molecules (the number depending on the size of the antigen molecule) are combined with one antigen molecule; the precipitate forms because such units stick to each other. The free antigen concentration is low in equilibrium with a precipitate of this type also.

No antigen-antibody precipitate is formed if antigen is present in great excess. Presumably in this case each divalent antibody molecule is combined with two antigen molecules. One of the antigen molecules is combined with the antibody molecule at the controlling site, and the other is combined with it at the catalytic site. Each antigen molecule is combined, however, with one antibody molecule only.

Proteins which may have a tendency to stick to gamma globulins may be co-precipitated when an antigen antibody precipitate is formed in their presence. An example for proteins which may be co-precipitated in this manner are the serum "complements"; which are "fixed" when an antigen-antibody precipitate is formed in their presence.

The Coupling Enzyme-Antigen System.—If the hapten $\bar{M}_j$ of the antigen P_j is a chemical analogue of the metabolite M_j, then the antigen can reversibly combine with the coupling enzyme C_j by virtue of the specific chemical affinity of the hapten to a part of the catalytic site of the coupling enzyme.

We postulate that the coupling enzymes resemble those antibodies of the rabbit which form a precipitate with antigen when the antibody is in excess. Accordingly, at low antigen concentrations a number of coupling enzyme molecules might be

combined with one antigen molecule and such units may then stick to each other and form a precipitate. We may assume that the free antigen concentration can be very low in equilibrium with such a precipitate.

The "Sensitive" Lymphatic Cells.—We assume that the cells of the lymphatic system go through a maturation process that carries the cell from its initial form, presumably the stem cell, to its mature form, presumably the plasma cell. When a lymphatic cell reaches a certain phase in this maturation process it becomes "sensitive" in the sense that it becomes capable of responding to the exposure to a soluble antigen with the formation of a specific antibody. We assume that what renders the cell sensitive, when it reaches this phase of its maturation process, is a general lowering of the repression factors to a value of perhaps $\lambda = 10^6$. (For definition of the repression factor see preceding paper.) This might be brought about by an increase in the activity of an enzyme which can universally hydrolize the R moieties of the repressors. In the preceding paper we have postulated the existence of such an enzyme and designated this hypothetical enzyme as the "hydrolase."

The set of constants assumed in the preceding paper, for the enzyme-forming system of the mammalian cell, we assume to hold for the antibody-forming system of the "sensitive" lymphatic cells. In the immature form of the lymphatic cells and in the rest of the somatic cells the concentration of the repressors ρ_0 and the corresponding repression factors λ presumably have a much higher value.

The Primary Response.—We assume that when a soluble antigen is injected intravenously or intraperitoneally into the rabbit, it penetrates into the cytoplasm of all the lymphatic cells. We further assume that if the antigen stays in the circulation long enough, the free antigen concentration in the lymphatic cells will be the same as the free antigen concentration in the circulation. This means that the chemical potential of the antigen inside the cytoplasm of the lymphatic cell is the same as the chemical potential of the antigen in the circulation.

According to the views here adopted, the primary response comes about in the following manner: when a soluble antigen P_j is injected into the rabbit it will diffuse into the lymphatic cells and precipitate the coupling enzyme C_j. As the result of this, the rate at which the repressor REP_j is formed will be reduced, and the concentration of this repressor will begin to fall. As the concentration of the repressor falls, the rate of formation of the antibodies A_j will increase.

Antibody molecules A_j present in the cell bind molecules of the repressor REP_j because of the specific affinity of the repressor molecule to their controlling site. Accordingly, in a sensitive cell, as the concentration of the antibody molecules A_j rises and the concentration of the repressor molecules REP_j falls, at some point in time the enzyme-forming system will lock and from then on the cell will produce the antibody molecules A_j at a high rate, even in the absence of antigen.

This sustained antibody production, by the cells in which the enzyme-forming system has locked in the primary response, represents a kind of memory, and it may account for the sustained immunity manifested by the rabbit.

Possibly a cell might lock simultaneously for the production of antibodies specific for two different haptens. But once a cell has locked for the production of an antibody, which is thereafter produced at a high rate, then subsequently a new stationary state establishes itself in the cell. The cell is then no longer "sensitive."

so that if it is exposed to another antigen it will not lock for the production of the corresponding antibody.

The Complexities of the Primary Response.—When the primary response is elicited in the rabbit by injecting an antigen which consists of a non-antigenic protein P_c to which are coupled m haptens $\bar{M}_j$, there will combine with an antigen molecule not only molecules of the coupling enzymes C_j but also molecules of the enzyme E_j and of the antibodies A_j.

The phenomena accompanying the primary response are even more complicated if the artificial hapten is coupled not to a protein which is non-antigenic in the rabbit but, for instance, to a foreign serum protein which *is* antigenic in the rabbit. When such an antigen diffuses into the lymphatic cells, various coupling enzymes corresponding to the various determinant groups carried by the antigen molecule, and also the corresponding enzymes E and antibodies A may combine with the antigen and form a precipitate.

On this basis it is possible to explain why a conjugated protein carrying an artificial hapten elicits more antibody directed against the artificial hapten if the protein is a foreign protein which is a good antigen in the rabbit, rather than if it is one of the rabbit's own serum proteins.

The Secondary Response.—We shall postulate that the cells of the lymphatic system contain a hypothetical enzyme S which, when present at high concentration, will inhibit cell division. We shall further postulate that this hypothetical enzyme S resembles complement, inasmuch as it can be co-precipitated if an antigen-antibody precipitate is formed in its presence.

When an antigen P_j is injected into a rabbit for the first time, a certain number of the sensitive lymphatic cells will lock and henceforth produce the antibodies A_j at a high rate. When a lymphatic cell locks and produces such proteins at a high rate, it will from then on produce most other proteins, including the hypothetical enzyme S, at a low rate. Thus, following the locking of the lymphatic cell in the primary response, the concentration of the enzyme S will fall, and it may reach a new stationary level within a few days or weeks.

If, about four weeks after the first injection of the antigen P_j, the same antigen is injected into the rabbit again, it will diffuse into the lymphatic cells and it will form a precipitate with the antibodies A_j in those lymphatic cells which have locked at the time of the first injection of the antigen. By the process of co-precipitation a certain quantity of the enzyme S will thereby be fixed, and, inasmuch as at the time of the second injection the level of this enzyme in the locked cells is low, the concentration of the enzyme may fall to the point where it can no longer inhibit cell division.

Thus, the second injection of the antigen will lead to proliferation of those cells which have locked at the time of the first injection of the antigen. This is our explanation of the secondary response.

If the views here adopted are correct, we may expect that the secondary response would be elicited by any compound carrying a hapten, which forms a precipitate with the antibody that is directed against this hapten, whether or not the compound is capable of eliciting the primary response.

The finding of compounds which are weak antigens, in the sense that they will

elicit only a weak primary response, but are good antigens, in the sense that they will elicit the full secondary response, would lend support to our theory.

A conjugated protein, obtained by coupling an artificial hapten to rabbit serum albumin, is supposed to be a very weak antigen in the rabbit, in the sense that it does not elicit in the primary response the formation of an appreciable quantity of antibody directed against the artificial hapten. On the other hand, a conjugated protein obtained by coupling an artificial hapten to a foreign serum globulin, which is antigenic in the rabbit, is supposed to be a very good antigen in the sense that it will elicit in the primary response the formation of a substantial quantity of antibody directed against the artificial hapten. (Oral communication, Herbert Anker, 1959.)

On this basis, our theory predicts that if we pre-immunize the rabbit with a conjugated foreign serum globulin and evoke the secondary response with the conjugated rabbit serum albumin, we should obtain in the secondary response a substantial quantity of antibody directed against the hapten. If, however, we pre-immunize the rabbit with the conjugated rabbit serum albumin and evoke the secondary response with the conjugated foreign serum globulin, then we should obtain in the secondary response a less substantial production of the antibody directed against the hapten.

In interpreting the results of an experiment of this type, one must keep in mind that when an antigen is injected for the second time one obtains not only a secondary response but also a "primary response," in which sensitive lymphatic cells will lock for the formation of the specific antibody. It should be possible, however, to distinguish these two responses, because the release of the antibody into the circulation from the secondary response presumably precedes its release from the "primary response."

The Decay of the Primary Response.—In the primary response the release of antibody into the circulation does not persist long at a substantial rate after the antigen has been eliminated from the circulation. It is conceivable, though by no means certain, that, as far as the release of antibody into the circulation is concerned, the phenomena which characterize the secondary response may play a part in the primary response also.

When a cell locks for the production of an antibody in the primary response the concentration of the antigen present in the circulation might be high enough to lead to the formation of an antibody-antigen precipitate in the locked cell and to a co-precipitation of the enzyme *S*. This might cause a proliferation of the locked cell as described above, in connection with the secondary response.

The locked cells which divide may disintegrate as fast as they are produced and release their antibody content into the circulation.

Soon after the antigen disappears from the circulation, this enforced proliferation of the locked cells may cease, but we may assume that the locked cells will keep on dividing, even though rather slowly, and releasing their protein content into the circulation. The amount of antibody produced which is specific for any given antigen may not be appreciable, but the total gamma globulin production of the adult animal may be a measure of the rate at which the locked lymphatic cells divide and disintegrate.

Newborn rabbits are not capable of forming antibodies and we may perhaps

assume that in the lymphatic cells of the newborn rabbit hydrolase activity is low, and therefore the repression factors λ are high. On this basis we may understand why lymphatic cells of the newborn rabbit cannot lock for the production of an antibody when they are exposed to an antigen.

The fact that in the young rabbit, just as in many other young animals, the rate of production of gamma globulin is low supports the view here adopted that the gamma globulins are produced by the locked lymphatic cells. The lymphatic cells which have not locked might not divide at all or might divide exceedingly slowly.

Immune Tolerance Induced in the Newborn Rabbit.—If a large amount of an antigen P_j is injected into a newborn rabbit, which cannot form antibodies, the antigen will diffuse into the lymphatic cells and there will be a condition of antigen excess both with respect to the coupling enzymes C_j and the antibodies A_j.

At the time, perhaps two weeks after birth, when some of the lymphatic cells of the rabbit become "sensitive" and capable of forming antibodies, the antigen concentration in the circulation will still be high.

The presence of a high concentration of the antigen P_j will prevent the locking of these cells with respect to the production of the antibodies A_j, because the haptens $\bar{M}_j$ of the antigen P_j compete with the repressor REP_j for the controlling site of the antibodies A_j.

The concentration of the antigen in the circulation of the rabbit will slowly fall and after several months it might reach a very low value. Nevertheless, the rabbit may still exhibit at that time specific immune tolerance with respect to the antigen. We may attempt to account for this phenomenon as follows:

If an antigen molecule is combined with the controlling site of the "attached" enzyme molecule we may assume that it will set up a steric hindrance and repress the formation of the antibody molecule in much the same way as would the specific repressor molecule.

We shall designate the enzyme-forming site which is specific for the formation of an antibody A_j as the ribosome B_j*. For the purpose of our discussion we may assume that corresponding to each gene G_j* which determines an antibody A_j there is present in the cell one and just one ribosome B_j*. Since we have assumed that there are a number of genes G_j* which correspond to the gene G_j, there will also be a number of ribosomes B_j* for each gene G_j. (The antibodies A_j which are made by the different ribosomes B_j* may differ somewhat from each other in their specific combining capacity with respect to the metabolite M_j and the hapten $\bar{M}_j$.)

We shall assume that each antibody A_j is formed inside the corresponding ribosome B_j*, and that the ribosome is a semi-closed structure.

We assume that water, salts, and small protein molecules, including the hypothetical enzyme U postulated in the preceding paper, may all diffuse freely in and out of the ribosome. The antibody molecule A_j which is produced in the ribosome can, however, not diffuse out of the ribosome, and its concentration will therefore rise until the osmotic pressure inside the ribosome exceeds the osmotic pressure in the cytoplasm outside of the ribosome. At that point water will begin to diffuse into the ribosome and as the hydrostatic pressure inside the ribosome increases, the ribosome may open up sufficiently to permit escape of a few antibody molecules. On the basis of such a model we may expect that even in cells which are not locked

for the production of an antibody each ribosome contains the antibody, for which it is specific, at a high concentration.

The concentration of the antigen injected into the newborn rabbit will remain high in the circulation for an extended period of time, and during this time enough antigen P_j will diffuse into the ribosome B_j^* to form an antigen-antibody precipitate. The "attached" antibody molecule A_j in the ribosome may be combined at its controlling site with an antigen molecule and may form part of the precipitate. Because the concentration of the free antigen in equilibrium with such a precipitate is low, the precipitate may still persist in the ribosome at the time when the concentration of the free antigen in the circulation has fallen so low as to be undetectable. In this manner we may account for enduring immune tolerance.

Accordingly, enduring immune tolerance requires the presence of the antigen in the lymphatic cells, but does not require the antigen to be present in the circulation at a detectable concentration.

Specific immune tolerance could not endure for long if the lymphatic cells of the rabbit which have *not* locked for the production of any antibody were to divide at a substantial rate, because new ribosomes B_j^* are presumably formed by the genes G_j^* when the cell divides, and antibody production in these new ribosomes would not be blocked. (Cells which have locked for the production of an antibody may be assumed to undergo divisions at an appreciable rate, but these cells are not capable of responding to the exposure of an antigen with the formation of the specific antibody, and would therefore not abolish immune tolerance.)

The Case of the X-Rayed Rabbit.—We may attribute the inability of the rabbit to respond with the formation of antibody, if the antigen is injected several days after the rabbit has been exposed to an X-ray dose of 400 r, to the inability of its lymphatic cells to lock for the formation of an antibody.

On this basis we may expect that, if a large quantity of an antigen is injected into the rabbit, several days after the rabbit has been exposed to an X-ray dose of 400 r the rabbit will exhibit enduring immune tolerance with respect to this antigen.

If an X-ray dose of 400 r is administered to the rabbit after the injection of an antigen, then by the time the X-ray exposure takes effect many of the lymphatic cells of the rabbit will have locked for the production of antibody directed against the antigen. These locked cells will proceed to form antibody, unaffected by the X-ray exposure. Accordingly, in this case the X-ray exposure will not prevent the rabbit from responding to the injection of the antigen with the formation of antibody.

Since the antibody released into the circulation in the secondary response is released by cells that have locked for the formation of the antibody at the time of the primary response, or by their descendants, there is no reason to expect that exposing the pre-immunized rabbit to an X-ray dose of about 400 r should inhibit the secondary response.

Concluding Remarks.—If the model for cellular differentiation, presented in the preceding paper, should in fact correctly describe the molecular basis of antibody formation, then we could be rather confident that the above given explanations for the major immunological phenomena are essentially correct. This holds in particular for the role we have attributed to the "locking" of sensitive lymphatic cells in the primary response and for the notion that the primary response and the second-

ary response are basically different phenomena. We cannot be equally confident, however, of the correctness of various assumptions which relate to details and which we introduced for the sake of the concreteness of the discussion. Among these are the assumptions which relate to the mechanisms by which the antigen lowers the repressor concentration and the assumptions which relate to the structure of the ribosomes B^*. As far as such details are concerned it might well be that our assumptions will have to be modified later on, in the light of future experimental data.

In the process of formulating the thoughts expressed in this paper I had the privilege of discussing the subject with Dr. Maurice S. Fox, The Rockefeller Institute, New York; Dr. Howard Green and Dr. Baruj Benacerraf, New York University College of Medicine; and Dr. Herbert Anker, The University of Chicago. It is a pleasure to acknowledge their helpful suggestions and criticism.

* This paper is the continuation of a paper entitled "The Control of the Formation of Specific Proteins in Bacteria and in Animal Cells," pages 277–292 of this issue of these PROCEEDINGS. The concepts, notations, and equations of the first paper carry over to this paper.

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*PURIFICATION OF A NERVE-GROWTH PROMOTING PROTEIN FROM THE MOUSE SALIVARY GLAND AND ITS NEURO-CYTOTOXIC ANTISERUM**

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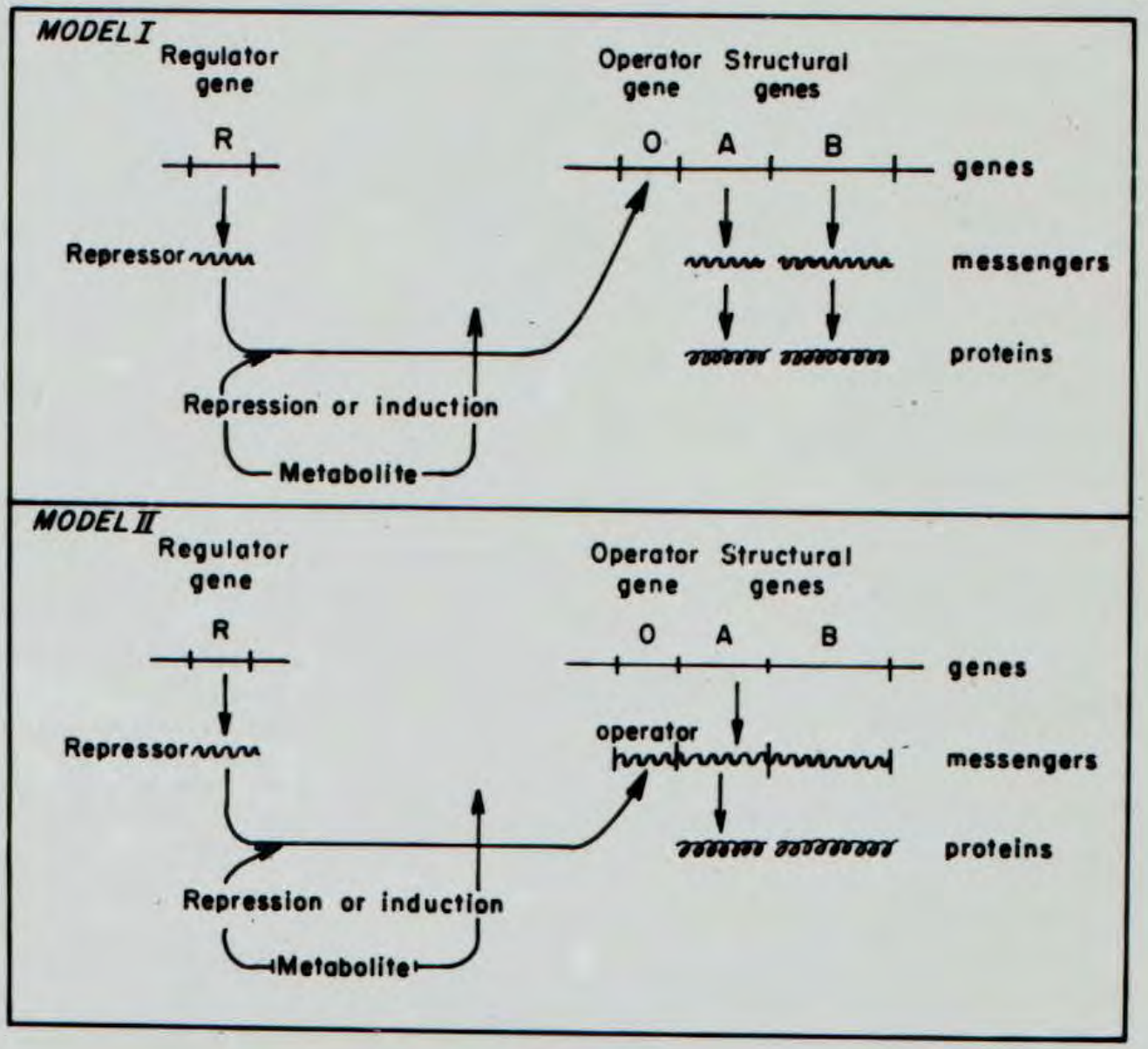
Introduction.—We have reported the isolation of a protein from snake venom which specifically promotes the growth of sensory and sympathetic nerve cells, both in tissue culture and in the living chick embryo.[1,2,3] The discovery of a similar factor in the salivary glands of the mouse, rat, and hamster,[1] extended the investigation to include mammals.

The first part of this paper is concerned with (*a*) the purification and nature of the nerve-growth factor in the submaxillary gland of the mouse, and (*b*) its effect, when injected into the mouse, on the gross chemical composition of the sympathetic ganglia.

In the course of this study we were able to prepare an antiserum to the purified growth factor isolated from the salivary gland. The antiserum inhibited, in tissue culture, the biological activity of the antigen. This gave us an opportunity to examine a question raised in our previous work concerning the possible physiological role (if any) of the growth factor in the normal organism. If a similar factor operates in the normal mouse, then the injection of the antiserum might interfere with its function. We have indeed found that the subcutaneous injection of this antiserum *in vivo* results in a specific atrophy and destruction of the sympathetic


MODEL I
Regulator gene
R
Repressor
Operator gene
Structural genes
O
A
B
genes
messengers
proteins
Repression or induction
Metabolite
MODEL II
Regulator gene
R
Repressor
Operator gene
Structural genes
O
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B
genes
operator
messengers
proteins
Repression or induction
Metabolite

Fig 6

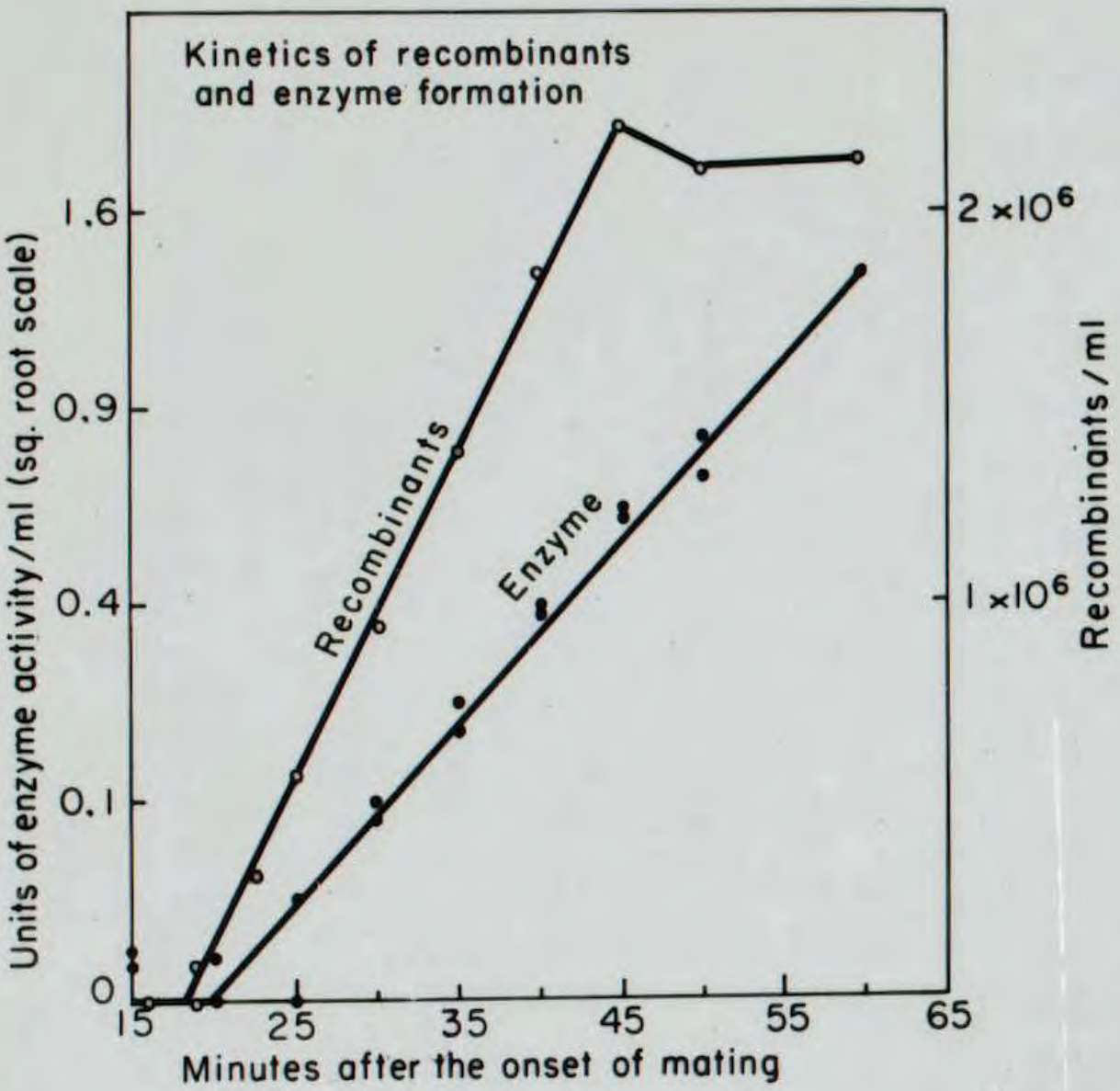
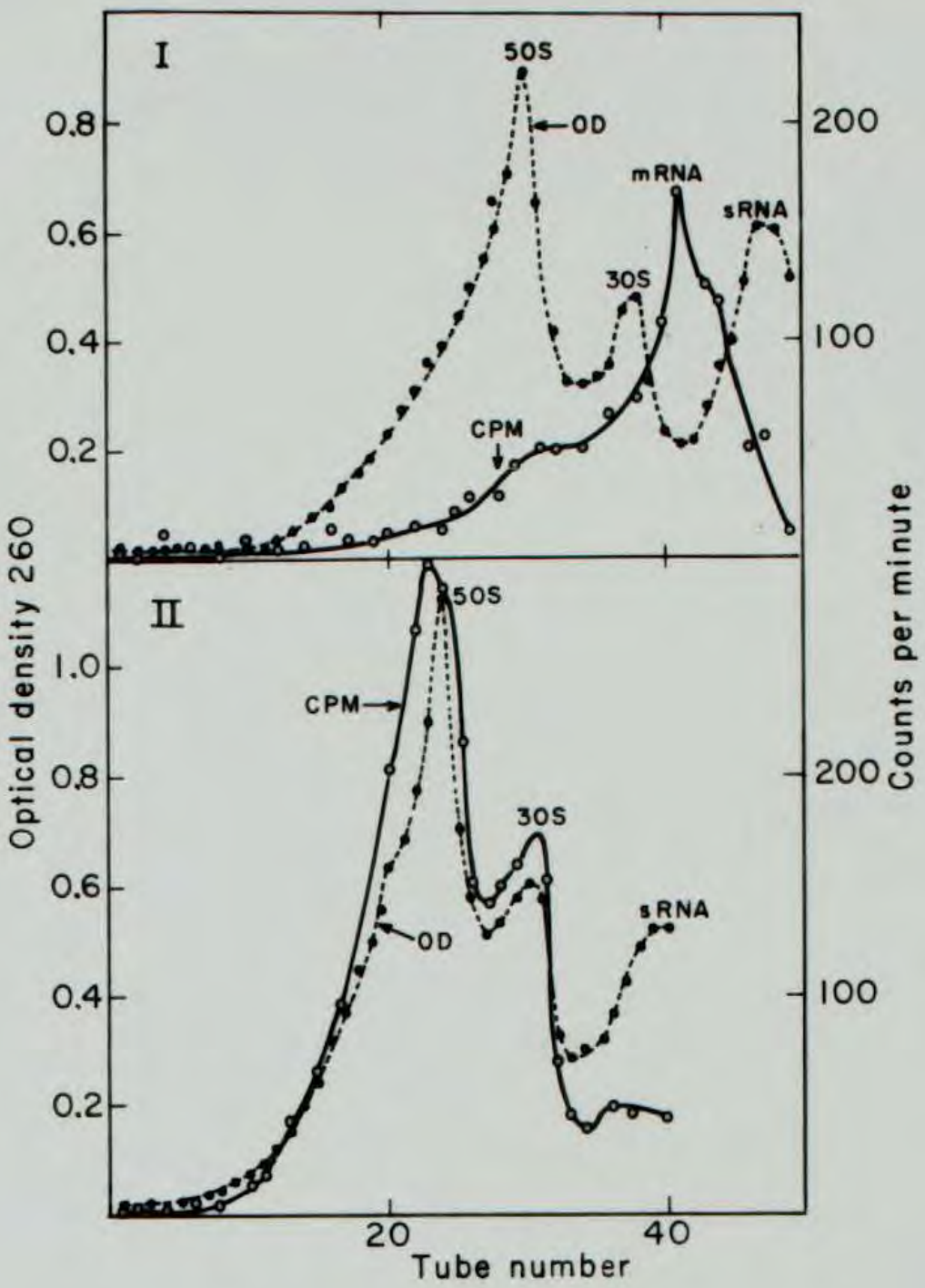

Kinetics of recombinants
and enzyme formation
Units of enzyme activity/ml (sq. root scale)
1.6
0.9
0.4
0.1
0
2 x10^6
1 x10^6
Recombinants / ml
Recombinants
Enzyme
15
25
35
45
55
65
Minutes after the onset of mating

Fig 7


I
50S
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mRNA
sRNA
30S
CPM
II
50S
CPM
30S
sRNA
OD
Optical density 260
Counts per minute
Tube number
0.8
0.6
0.4
0.2
1.0
200
100
20
40

Fig A

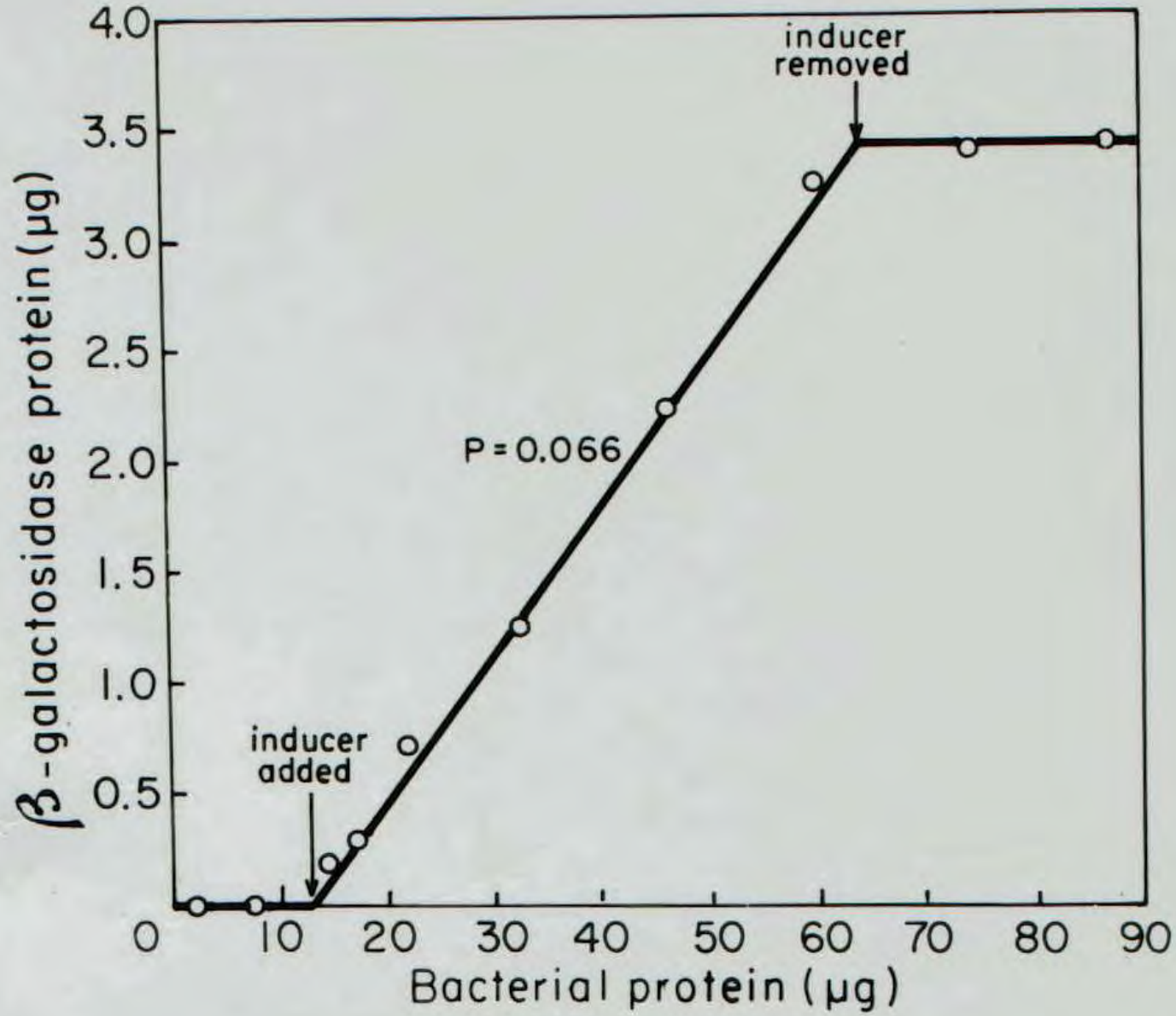

4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
β-galactosidase protein (μg)
inducer removed
P = 0.066
inducer added
10
20
30
40
50
60
70
80
90
Bacterial protein (μg)

Fig-1

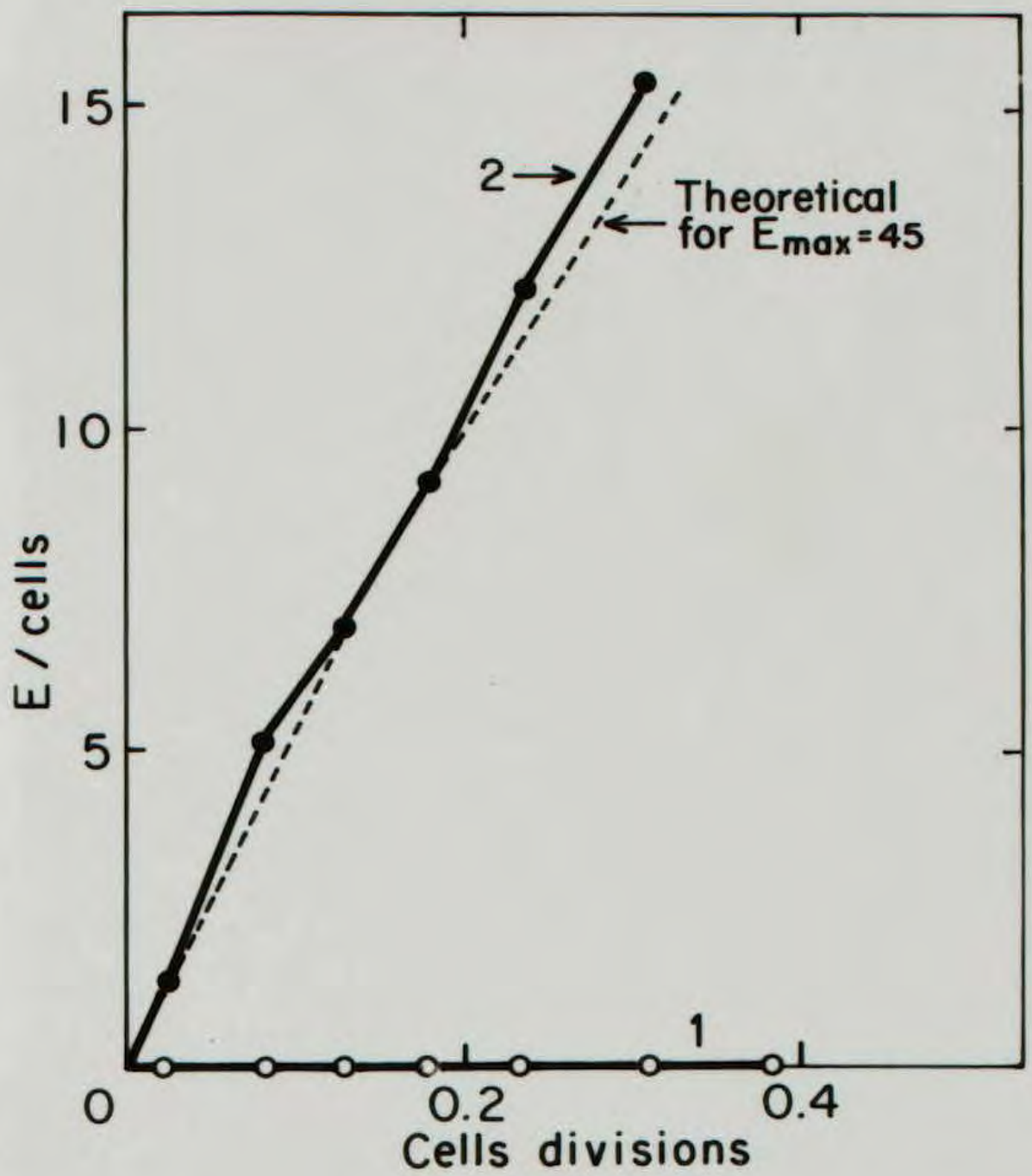

15
10
5
0
E / cells
2
Theoretical
for $E_{max}=45$
1
0.2
0.4
Cells divisions

Fig 2

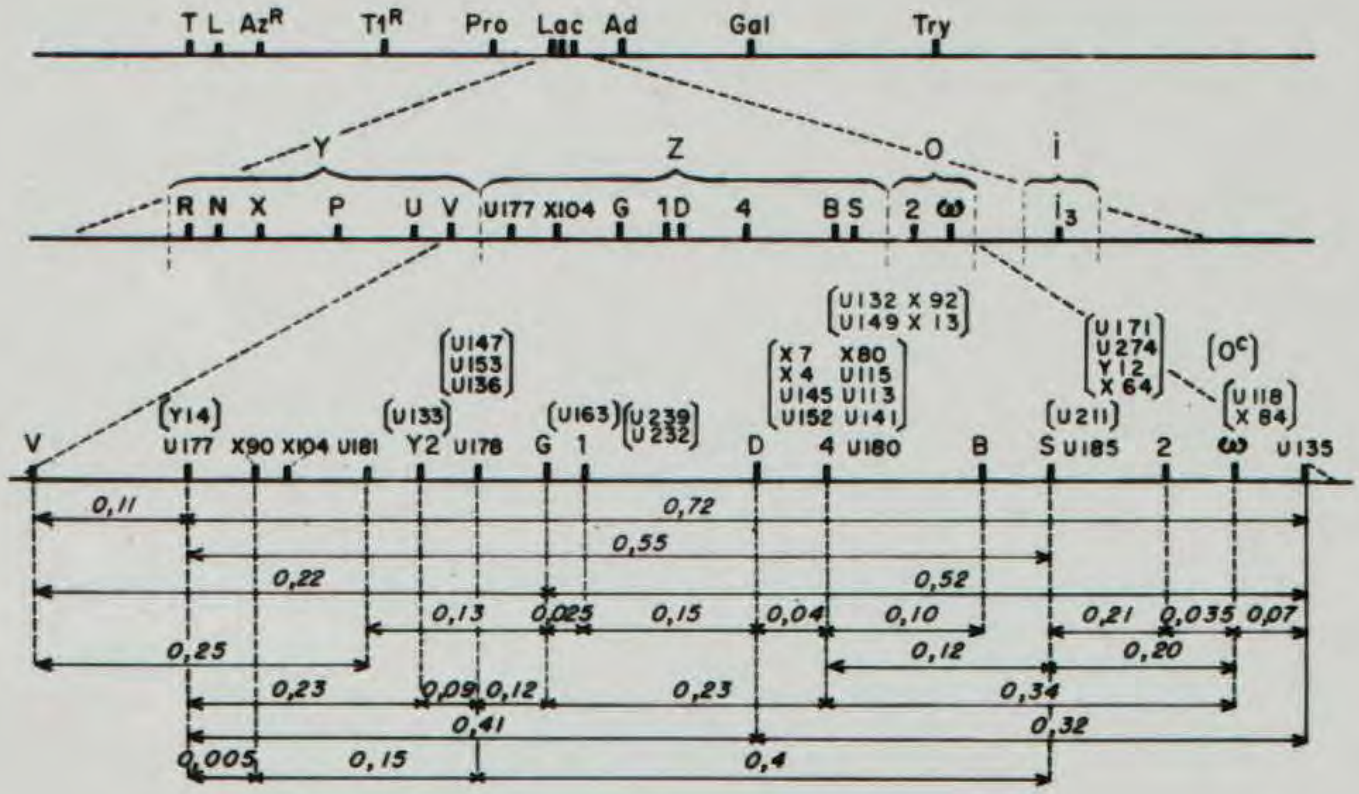

T L Az^R T1^R Pro Lac Ad Gal Try
Y
Z
O
i
R N X P U V U177 X104 G 1D 4 B S 2 ω i3
(U132 X 92) (U149 X 13)
(U147) (U153) (U136)
(X7 X80) (X4 U115) (U145 U113) (U152 U141)
(U171) (U274) (Y12) (X64)
(O^c)
(Y14)
(U133)
(U163) (U239) (U232)
(U118) (X84)
(U211)
V U177 X90 X104 U181 Y2 U178 G 1 D 4 U180 B S U185 2 ω U135
0,11
0,72
0,55
0,22
0,52
0,13 0,025 0,15 0,04 0,10 0,21 0,035 0,07
0,25
0,12
0,20
0,23 0,09 0,12 0,23 0,34
0,41
0,32
0,005 0,15 0,4

Fig 3

Froom book -- insert to pp. 8 and 9 -- second draft

Where Δ represents the average time which an enzyme formed will remain sitting on the paragene.

Δ is given by

(1a) $$\Delta = \tau(E\text{-}M^*)\frac{1}{p(M^*)}$$

where $p(M^*)$ represents the probability that an enzyme molecule sitting on the paragene is complexed by a molecule of the inducer, M*, and $1/\tau(E\text{-}M)$ represents the rate at which the enzyme-inducer complex dissociates from the paragene. As may be seen, Δ does not depend on $\tau(AA)$ and is only a function of the inducer concentration, M*.

For the rate of production of the enzyme, N, divided by τ_{gen}, we may write

(1b) $$\text{rate} = \frac{N}{\tau_g} = \frac{1}{\Delta + \tau(AA)}$$

We may now see how, according to formula (1b), the ratio of the quantities of two different enzymes, maintained in the steady state of the growing culture, will change according to (1b), when we slow the growth rate of bacteria which require an amino acid by increasing $\tau(AA)$ through the lowering of the concentration of that amino acid. When the concentration of that amino acid is high, so that the bacteria grow fast, we have

(1c) $$\frac{N_1(\text{fast})}{N_2(\text{fast})} = \frac{\Delta(1) + \tau(AA;\text{fast})}{\Delta(2) + \tau(AA;\text{fast})}$$

We shall now consider the case when at such fast growth, we have

$$\Delta(2) >>> \Delta 1$$

(1d) $$\Delta(2) >>> \tau(AA, fast)$$

i.e. and we have $\frac{N_1(fast)}{N_2(fast)} >>> 1$

so that N(E-1) is very highly induced compared to E-2. And now we shall consider how the ratio N_1/N_2 must change according to equation (1c) when we increase τ(AA) by slowing the growth of the bacteria to the point where the rate of production of the enzyme, E-2, is down by a factor of 2. In order to reach this point we must make τ(AA) sufficiently large so that we have KX in equation (16)

(1e) $$\tau(AA; slow) = \Delta(2)$$

In this case we obtain

$$\frac{N_2(slow)}{\tau_g} = \frac{1}{2\Delta(2)}$$

(1g) $$\frac{N_1(slow)}{\tau_g} = \frac{1}{\Delta(2)}$$

and from this we obtain

(1h) $$\frac{N_1}{N_2} = 2$$

As we may thus see, equation (1) says that if at a fast growth rate enzyme 1 is produced at a much higher rate than enzyme 2, when we grow the bacteria more slowly so that the rate of production of enzyme 2 is half, the bacteria maintain of the more abundant enzyme, E-1, only

twice as many molecules per cell as of the less abundant enzyme, E-2.

This is a flagrant violation of the principle of growth-rate independence.

To propose [illegible]
[illegible] Kuhn
[illegible].

$$\frac{dx}{dt} = -\alpha x - AM^* x + \beta y \qquad x_0 = 1$$

$$\frac{dy}{dt} = AM^* x - \beta y - \alpha y \qquad y = 0$$

Then $$\frac{d(x+y)}{dt} = -\alpha(x+y) \quad \text{with} \quad (x+y)_0 = 1.$$

$$\therefore \quad x + y = e^{-\alpha t}$$

x uncombined
y combined with M^*

Then $$\frac{dx}{dt} = -(\alpha + \beta + AM^*)x + \beta e^{-\alpha t}$$

Assume $\beta + AM^* \neq 0$.

Then $$x = \frac{\beta}{\beta + AM^*} e^{-\alpha t} + \frac{AM^*}{\beta + AM^*} e^{-(\alpha+\beta+AM^*)t}$$

$$\frac{p}{\alpha} = \int_0^\infty x\,dt = \left[\frac{\beta e^{-\alpha t}}{-\alpha(\beta + AM^*)} + \frac{AM^* e^{-(\alpha+\beta+AM^*)t}}{-(\alpha+\beta+AM^*)(\beta+AM^*)} \right]_0^\infty$$

$$\frac{p}{\alpha} = \frac{\beta}{\alpha(\beta+AM^*)} + \frac{AM^*}{(\alpha+\beta+AM^*)(\beta+AM^*)}$$ assuming $\alpha > 0$, $\alpha + \beta + AM^* > 0$

$$\frac{p}{\alpha} = \frac{\alpha+\beta}{\alpha(\alpha+\beta+AM^*)}$$

$$y^* = \frac{\alpha+\beta}{\alpha+\beta+AM^*}$$

fraction free [~~[illegible]~~]

$$p = \frac{\frac{\alpha}{\beta} + 1}{\frac{\alpha}{\beta} + 1 + \frac{AM^*}{\beta}}$$

~~If $\alpha = 0$, $\beta > 0$ then $\int_0^\infty x\,dt = \infty$~~

~~If $\alpha = \beta = 0$ then $\int_0^\infty x\,dt = \frac{1}{AM^*}$.~~

~~$\alpha > \beta$~~
~~$\beta < \alpha$~~

Important

If $\beta \gg \alpha$

$$p \cong \frac{1}{1 + \frac{M^*}{k^*}}$$ as it should be

$\beta > AM$

$\beta < AM$

$\frac{1}{\beta} = \tau(M) < \frac{1}{AM}$ ~~β~~

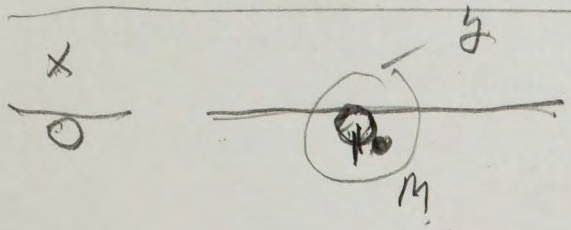
$\frac{1}{\beta} = \tau(M) > \frac{1}{AM}$

$\frac{1}{\beta} = \tau(M) > \tau(E) = \frac{1}{\alpha}$

$\alpha > \beta$

$y^* = \frac{y}{\frac{1}{\alpha}}$

$y^* = \frac{\alpha + \beta}{\alpha + \beta + AM^*}$



$x = -\alpha - AM + \beta y$

$y = AM - \beta y - \alpha y$

$e^{-\alpha}$

$\tau(E) < \tau(M)$

$y^* = \frac{1}{1 + \frac{M}{K}\frac{\tau(E)}{\tau(M)}}$

Possible

~~$AM \times \tau(E)$~~

~~$y^* = AM \times \tau(E)$~~

$y^* = \frac{1}{\alpha + AM^*}$

$= \frac{1}{1 + AM\tau(E)}$

Peut $\alpha < \beta$

$\tau(E) > \tau(M)$

$y^* = \frac{\beta}{\beta + AM^*} = \frac{1}{1 + \frac{AM^*}{\beta}\tau(M)}$

$= \frac{1}{1 + \frac{M}{K}}$

fraction of E sitting on porosphere is free from M^* (time τE during which)

$$y^* = \frac{1 + \frac{\beta}{\alpha}}{1 + \frac{\beta}{\alpha} + M^*} = \frac{1+\frac{\beta}{\alpha}}{1+\frac{\beta}{\alpha} + L A M \tau_E}$$

$$\frac{1}{y^*} = 1 + \frac{\frac{M}{K}\frac{\tau(E)}{\tau(M)}}{1 + \frac{\tau(E)}{\tau M}}$$

~~$\frac{1}{y^*} = 1 + \frac{\frac{M}{K}}{\tau(M) + \tau(E)}$~~

~~$y^* = \frac{\tau(M) + \tau(E) + \frac{M}{K}}{\tau(M) + \tau(E)}$~~

$$\frac{1}{y^*} = \frac{1 + \left(\frac{M}{K} + 1\right)\frac{\tau(E)}{\tau(M)}}{1 + \frac{\tau(E)}{\tau M}}$$

$$y^* = \frac{1 + \tau(E)/\tau(M)}{1 + \left(\frac{M}{K} + 1\right)\frac{\tau(E)}{\tau(M)}}$$

for $\tau(E)/\tau(M) >> 1$ or $\tau(E) >> \tau(M)$

$$y^* = \frac{1}{1 + \frac{M}{K}} = \frac{1}{1 + A M \tau(M)}$$

for $\tau(E)/\tau(M) \ll 1$; $\tau(E) < \tau(M)$

$$y^* = \frac{1}{1 + \frac{\tau(E)}{\tau(M)} + \frac{M}{K}\frac{\tau(E)}{\tau(M)}}$$

$\frac{M}{K}\frac{\tau(E)}{\tau(M)} \ll 1$

This is smaller in terms of K

limit:

$$y^* = \frac{1}{1 + A_M \tau(E)}$$

General

$$y^* = \frac{1 + \tau(E)/\tau(M)}{1 + A_M M \tau(E) + \tau(E)/\tau(M)}$$

with no M term

$$\frac{1}{y^*} = 1 + \frac{A_M M \tau(E)}{1 + \tau(E)/\tau(M)} = 1 + \frac{A_M M \tau(E)\tau(M)}{\tau(E) + \tau(M)}$$

~~with throughput~~

$$\underbrace{\tau(E) A_R R}\ y^* = A_R R \left(\frac{\tau(E)}{1 + \frac{A_M M \tau(E)\tau M}{\tau(E) + \tau(M)}} \right) =$$

$$= \frac{A_R}{R}\left(\frac{1}{\tau(E)}\right) \qquad \tau(E) A_n R \gamma^*$$

$$= \frac{A_R}{R}\frac{K}{K} \times \frac{\tau(E)}{\tau(M)} \frac{1}{1 + \frac{\tau(E)}{\tau(E)+\tau(M)} \frac{M}{K}}$$

$$\tau(E) A_n R \gamma^* = \frac{R}{K} \frac{\tau(E)}{\tau(M)} \frac{1}{1 + \frac{M}{K} \frac{\tau(E)}{\tau(E)+\tau(M)}}$$

1) $\frac{dN}{dt} = \frac{1}{\Delta} - \frac{N}{\tau_0}$ $\qquad N_\infty = \frac{\tau_0}{\Delta}$

2) $\frac{dN}{dt} = \frac{1}{\Delta + \Delta(1 - e^{-\frac{t}{2\tau_0}})} - \frac{N}{2\tau_0}$ $\qquad N_0 = \frac{\tau_0}{\Delta} = N_\infty$

3) $\frac{dN}{dt} = \frac{1}{\Delta + \Delta e^{-\frac{t}{\tau_0}}} - \frac{N}{\tau_0}$ $\qquad N_0 = N_\infty = \frac{\tau_0}{\Delta}$.

Stage 2). Solution of equation 2) is given by:

$$N = N_0 e^{-\frac{t}{2\tau_0}} + \frac{\tau_0}{\Delta}\left[1 - e^{-\frac{t}{2\tau_0}} + \frac{1}{2} e^{-\frac{t}{2\tau_0}} \log\left(2e^{\frac{t}{2\tau_0}} - 1\right)\right]$$

$$= \frac{\tau_0}{\Delta}\left[1 + \frac{1}{2} e^{-\frac{t}{2\tau_0}} \log\left(2e^{\frac{t}{2\tau_0}} - 1\right)\right]$$

N rises initially, reaches maximum at $t = 1.6623\,\tau_0$, then falls to N_∞.

$$N_{Max} = N_{1.6623\,\tau_0} = \frac{\tau_0}{\Delta}(1 + 0.2784).$$

Stage 3) Solution of equation 3) is given by:

$$N = N_0 e^{-\frac{t}{\tau_0}} + \frac{\tau_0}{\Delta}\left[1 - e^{-\frac{t}{\tau_0}} - e^{-\frac{t}{\tau_0}} \log \frac{e^{\frac{t}{\tau_0}} + 1}{2}\right]$$

$$= \frac{\tau_0}{\Delta}\left[1 - e^{-\frac{t}{\tau_0}} \log \frac{e^{\frac{t}{\tau_0}} + 1}{2}\right]$$

N falls initially, reaches minimum at $t = 1.1976\,\tau_0$, then ~~falls~~ rises to N_∞.

$$N_{Min} = N(t = 1.1976\,\tau_0) = \frac{\tau_0}{\Delta}(1 - 0.2318)$$

Transition of
induced current level
when tyu is shifted.

Hirondo Kuhi
Sept 23 rd / 57

trolled the muscle tend to recapture control in regeneration. Such selective reaffiliation of nerve and muscle indicates that some chemical specificity must match one to the other.

Selective outgrowth of regenerating nerves to their proper end-organs seems not to be the rule, however, even in lower forms. Among mammals it has not been found at all, except on the much more gross scale that differentiates sensory from motor endings, smooth muscle from striated muscle, muscle from gland, and so on. Nor does simple selective outgrowth account for the restoration of function in salamanders. The early studies by Weiss showed that fiber outgrowth and muscle re-innervation generally proceed in these animals in a random, nonselective manner, comparable to that in mammals. Upon re-innervation, however, salamander muscles regain their former coordination and timing, even when their function is disoriented by nerve-crossing.

These observations suggest that the rearrangement of connections in the periphery of the salamander nervous system has chemical repercussions that result in a compensatory shift of reflex relations at the centers. It is postulated that the motor-nerve cells regenerating into new muscles take on a new chemical flavor, as it were. Thereupon their old central associations dissolve, and new ones form to match the new terminals in the periphery. The reflex circuit would thus be restored to its original state, with the peripheral and central terminals linked by a new pathway. Higher animals, lacking this embryonic type of structural plasticity, show no restoration of function.

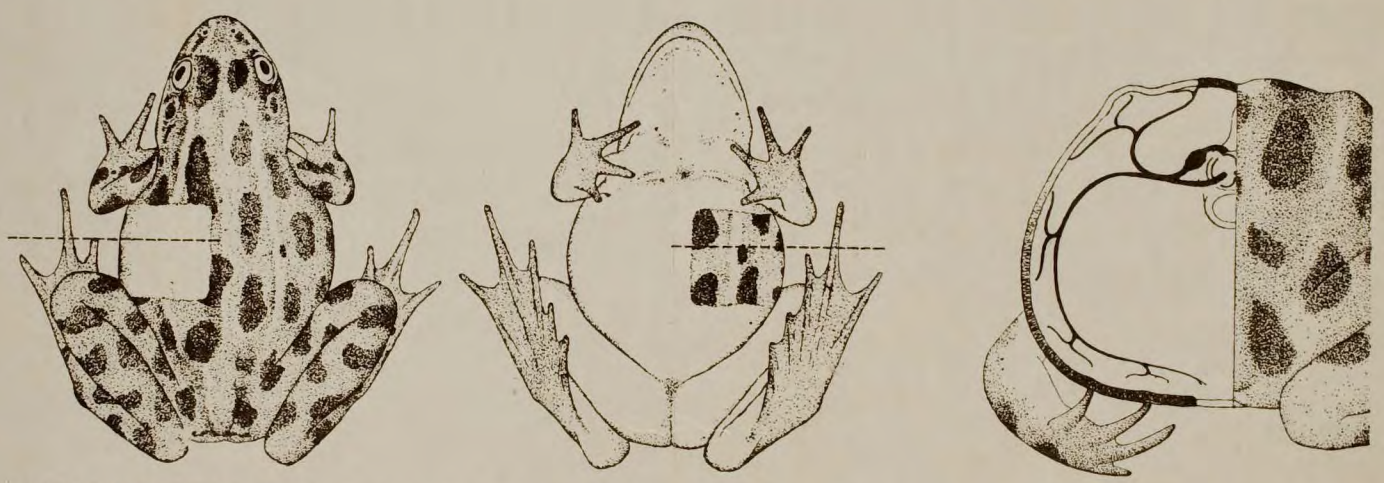
This explanation at first seemed rather far-fetched, especially from the standpoint of electrophysiology, which offers no evidence for such qualitative specificity among nerve fibers. However, the underlying idea is well supported by recent experiments on the regeneration of sensory nerves.

At the University of Chicago Nancy M. Miner, one of my former associates, is responsible for a significant series of experiments indicating the role of some sort of chemical specificity in the hookup of the nervous system. She grafted extra hindlimb buds onto the backs of tadpoles; the buds became connected to the sensory fibers that would normally innervate the skin of the belly, flank and back [*see illustration on preceding page*]. The grafted leg served only as a sensory field for the nearby sensory nerves because there are no nearby limb nerves to invade it. When a stimulus was applied to the grafted limb in the mature frog, the animal moved the normal hindlimb on the same side, just as it would if the normal limb had received the stimulus. The belly and trunk nerves connected to the grafted limb had evidently taken on a hindlimb "flavor" and then formed the appropriate reflex connections in the central nervous system. In another experiment Miner removed a strip of skin from the trunk of a tadpole, cut its nerves and replaced it so that the skin of the back now covered the belly, and vice versa [*see illustration below*]. When the grown frogs were stimulated in the grafted area of the back, they responded by wiping at the belly with the forelimb; when they were stimulated in the grafted area on the belly they wiped at the back with the hindlimb.

To account for these experimental findings it is necessary to conclude that the sensory fibers that made connections to the grafted tissues must have been modified by the character of these tissues. It is therefore unnecessary to postulate that each nerve fiber in embryonic development makes some predestined contact with a particular terminal point in the skin. Growing freely into the nearest area not yet innervated, the fibers establish their peripheral terminals at random. Thereafter they must proceed to form central hookups appropriate for the particular kind of skin to which they have become attached. It seems clearly to be some quality in the skin at the outer end of the circuit that determines the pattern of the reflex connections established at the center.

No attraction from a distance need be invoked in this selective patterning of the central hookup. The multiple branches of each nerve fiber undergo extensive ramification among the central nerve cells, with the tips of the branches making numerous contacts with all the cells in the vicinity. Presumably most of the contacts do not affect the growing fiber-tips. It is only when contact is made with central nerve cells which have the appropriate chemical specificity that the growing fiber adheres and forms the specialized synaptic ending capable of transmitting the nerve impulse.

In man these observations and interpretations provide the basis for the new



ROTATED PATCH OF SKIN demonstrates how embryonic nerves respond to the biochemical "flavor" of the tissues they innervate. Here a strip of skin was removed from a tadpole, cut free of all connections, and replaced so that the skin of the back now covered the belly and vice versa. In the grown frog the skin retained its original color and flavor despite its location, as shown in the dorsal and ventral views (*left and center, respectively*). The cutaway view at right (*made along broken lines*) shows how new nerves have invaded the graft and formed spinal reflex-arcs appropriate to the skin's flavor rather than to its location. Thus when the belly skin on the back is stimulated, the frog wipes at its belly; when the back graft on the belly is stimulated, the frog wipes at its back.

Insert on page 7 B

Except in the case of certain mutant bacteria discussed below, we may assume that we have in general in bacterial cells

(7) ~~(10)~~ $\rho_0 \gg Z_{oo}$

and accordingly we

(8) ~~(11)~~ $\rho = \rho_0$

Therefore, in bacterial cells we may in general write (12) in the place of (11) so that we have

(12) rate $= \frac{1}{\tau_E} \frac{1}{\frac{\rho_0}{K} P + 1}$

Accordingly ~~in general~~ the rate of formation of the enzyme will be ~~independent of the amount of enzyme present in the cell.~~

It may be seen from (12), ~~also~~ that if ~~presence of the~~ inducer $\bar{M}$ does not effect the ~~concentration~~ ρ_0 of the repressor molecules in the cell, then the rate of formation of the enzyme cannot rise ~~faster~~ than linearly with ~~it, at~~ the intracellular concentration of the inducer.

One must not assume, ~~of course, that for the boostable enzymes~~ the enzyme concentration ~~rates the value Z~~ when the repressor concentration falls to 0. ~~For~~ the boostability of the enzyme ~~might well~~ be a "limited boostability" in the sense that when the enzyme concentration becomes high, some factor other than the repressor concentration will limit the rate at which the enzyme is produced. It might well be to keep this in mind ~~particularly~~ when we apply the above quoted equations to mammalian cells.

The Productions of the Model.

On the basis of the model described by the above given euqations we may expect the following:

1) If the cells of a bacterial strain are incapable of converting a close chemical analogue $\bar{M}$ of ~~it~~ the repressors' metabolite moity M into this metabolite moity, then ~~(in general)~~

Unedited version.

October 14, 1959.

The repression of enzymes in bacteria, the molecular basis of cellular differentiation and the formation of antibodies in mammals

by

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That an amino-acid may repress the formation of an enzyme involved in its synthesis was first shown by Monod and Cohen-Bazire (1953) as well as Cohn, Cohen and Monod (1953). Four years later H. J. Vogel (1957) found that arginine, when added to a growing bacterial culture, will repress the formation of acetyle ornithase, one of the early enzymes along the biochemical pathway leading to arginine. This observation provided rather strong circumstantial evidence in favour 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, might 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 enzymes which are involved in the formation of the repressor. Thus the inducer would enhance the formation of an enzyme 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 the induction must be considered in conjunction with the biochemical pathway leading from galactose to glucose -1-P. (Oral Communication January 1957).

* The author wishes to express his gratitude for the support he received while serving as a consultant to the Basic Research Program of the National Institute of Health, U.S. Public Health Service.

The induction of β-galactosidase in a culture of bacteria growing ~~with~~ on succinate or lactate, as the carbon source, is inhibited by adding glucose to the growing culture. We may therefore assume that an intermediate metabolite in the sequence of metabolites lying between galactose and glucose 1-P ~~is~~ as the precursor of the repressor. The induction of β-galactosidase by certain galactosides in a culture of bacteria growing ~~in~~ on lactate ~~of~~ or succinate ~~as the carbon source~~, may then be explained on the basis of the suggestion made by Werner Maas ~~by assuming that~~ and has my help such a galactoside inhibits enzymes which lie on the biochemical pathway between glucose-1-P and the intermediate metabolite which is the precursor of the repressor. I believe that ~~this assumption is in fact correct~~ such an inhibition does in fact take place and that it may explain why the rate of the formation of β-galactosidase rises faster than linearly with the ~~inducer~~ intracellular concentration of the inducing galactoside within the bacterial cell.

It ~~is~~, however, ~~not possible~~ unable to explain on ~~the same~~ a similar basis ~~the phenomena of~~ the induction of ~~the~~ enzymes by ~~its~~ their substrate, in the ~~comparatively simple case~~ large class of the degradative enzymes, such as for instance the enzymes which degrade tryptophane in pseudomonas fluorescnence.

Therefore, I was led to assume that, in general, the inducer ~~must do more~~ may also do something else than just inhibit the formation of a repressor. Specifically I was led to believe that the repressor may reduce the rate of formation of the enzyme by combining at a certain ~~site~~ locus, the controlling site of the enzyme, with ~~the~~ an enzyme molecule which is still attached to its enzyme-forming site and may thereby prevent the attached enzyme molecule from leaving its enzyme-forming site.

~~The~~ An inducer may then enhance the formation of enzymes by competing with the repressor for the controlling site of the "attached" enzyme molecule.

I was further led to believe that the repressor of the enzyme β-galactosidase ~~is~~ may be a molecule composed of two moities. One of these (which we may call the metabolite moity) may be a

galactoside and the other (which we may call the R moity) might perhaps be a polynucleotide. Certain galactosides may then enhance the formation of the enzyme β-galactosidase by competing for the controlling site of the attached enzyme molecule with the metabolite moity 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 also in lectures I gave in Europe and America in the subsequent six months.

This model appears to be capable of accounting not only for enzyme repression and induction but also for a number of immunological phenomena displayed by the rabbit. It was not able, however, to furnish, at that time, a simple and convincing explanation for the phenomenon of lasting immunity which manifests itself in the secondary antibody response which may be elicited in the rabbit when it is given an injection of an antigen by which it has been immunized before. 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 interval and thus involves a "memory" which fades away only very slowly.

Two years ago, when the model was first presented it was supported only by scattered experimental facts. In particular the tenet that the R moity 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 mainly due to experiments of George Cohen, François Jacob, Jacques Monod and Arthur Pardee carried out at Institut Pasteur in Paris. These experiments give strong support to the belief that enzyme repression may be the key to the understanding of the induction of the enzyme β galactosidase. Moreover, one of these experiments shows that the bacterial gene which is responsible for the repression of the formation of β-galactosidase acts, not by causing the synthesis of an enzyme, but

rather by causing the synthesis of a molecule which is not a protein. What the experiment actually shows is, that adding chloramphenicol to a growing bacterium does not prevent the gene, when it is introduced into this bacterium, from causing the repression of the formation of β-galactosidase. This remarkable finding is consistant with the notion that this gene may be exerting its effect, by causing the formation of the R moity of the repressor of β galactosidase and that this R moity might be a polynucleotide.

As far as I can see the results of all the newer experiments are so far still consistent with the model that was formulated two years ago. However, it remains to be seen whether future experiments will confirm one of the assumptions of the model, i.e. the assumption that the repressor controls the rate of enzyme formation at the level of the enzyme forming site, which might be a ribosome, rather than at the level of the gene, one step removed.

The present communication is mainly concerned with the problem of antibody formation in mammals, such as for instance the rabbit. I therefore propose to dwell here only rather briefly on the phenomenon of enzyme repression in bacteria and its interpretation in terms of the model. The interpretation of antibody formation in the rabbit I propose however to discuss in greater detail, on the basis of the model.

On this occasion I shall present a novel consideration which may account for the phenomenon of lasting immunity exhibited by the rabbit. This consideration may provide the molecular basis for the sustained production of an antibody subsequent to the first injection of the antigen, for which the antibody is specific. It might conceivably also provide the molecular basis for cell differentiation, in general.

Repression and induction of the formation of enzymes in bacteria.

1) Enzymes leading to glucose-1-P.

There are a number of inducible 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 inconsistent with the views on enzyme formation and may be readily understood on the basis of considerations presented by Neidhardt and Magasanik (October 1956).

I do not propose to discuss here any of the enzymes which fall into this general class, however, even though the best studied enzyme, β-galactosidase appears to fall into this class. I rather propose to discuss here two other large classes of enzymes which we may designate as "anabolic" enzymes and "degradative" enzymes.

2) Anabolic Enzymes.

The enzymes which lie along biochemical pathways that lead to the formation of an indispensable building block of the cell, such as an amino-acid, purine or pyrimidine, we may call "anabolic" enzymes. The repression of these enzymes has been demonstrated by Vogel -The enzymes involved in the biosynthesis of the amino-acid arginine may be representative for enzymes of this class.

The formation of a number of enzymes E_k along the biochemical pathway leading to arginine may be repressed by adding arginine to a growing bacterial culture. I do not assume that the chemical molecule responsible for the repression of the formation of these enzymes is arginine itself, but rather that the repressor, REP (E_k) is a composite molecule consisting of a metabolite moity, arginine, and another moity designed by R_k (arg). Thus we may write for the chemical formula of the repressor REP (E_k)

$$REP(E_k) = Arginine - R_k$$

As stated above the R moity of such a repressor molecule

(Arthur Pardee, Werner Maas and Luigi Gorini and Bruce Ames, Magasanik

P.T.O to 5a

2) Special anabolic enzymes.

The enzymes which lie along the biochemical pathways that lead to the formation of an aminoacid, purine or pyrimidine we may call "special anabolic enzymes". We may take the enzymes involved in the biosynthesis of the aminoacid arginine to be representative for the enzymes of this class.

One of these enzymes, ornithin transcarbamylase which converts ornithine into citrulline, has been studied by Luigi Gorini and Bernard Maas. In a certain stain of coli the formation of this enzyme may be repressed by adding arginine to a crown bacteria culture. If the intracellular concentration of arginine is lowered in the bacterium by growing the bacterium in a chemostat (with arginine as a controlling growth factor), the rate of production of the enzyme rises by a factor of about 25.

There are other related strains of coli in which the rate of enzyme formation can not be thus boosted by lowering the intracellular concentration of arginine. We shall leave such unboostable strains out of consideration on this occasion.

We do not assume that the chemical molecule responsible for the repression of this enzyme - in the "boostable" strains - is arginine itself, I rather assume that the repressor is a composite molecule consisting of two moities. One of these is arginine, to which we may refer as the "metabolite" moity, and the other is a moity which we may designate by R_K to which we shall refer as the R-moity. Thus we may write for the chemical formula on the repressor REP_K.

REP_K = arginine - R_K

As stated above the R-moity of such a repressor molecule

might be a polynucleotide.

A model which may be capable of accounting for the repression of the formation of enzymes is outlined in the synthesis of arginine is as follows:

The enzymes lying along the biochemical pathway leading to arginine have two sites, the "catalytic" site and the "controlling" site. The substrate of the enzyme has a specific chemical affinity to the catalytic site, whereas the arginine moity of the repressor has a specific chemical affinity for the "controlling" site.

The model assumes that the polypeptide chain of the enzyme molecule is synthetised along a specific enzyme forming site, presumably a ribosome, which determines the amino acid sequence of the polypeptide. This polypeptide folds up to form the enzyme molecule, but the molecule remains attached, perhaps through a covalent bond to the enzyme forming site. If there are repressor molecules present in the cell, a repressor molecule may combine reversibly with the attached enzyme molecule by virtue of the specific chemical affinity of the arginine moity of the repressor to the controlling site of the attached enzyme molecule. An attached enzyme molecule which is so combined with a repressor molecule can not leave its enzyme-forming site, and accordingly the formation of the enzyme is repressed.

Pour fixer les idées concerning the mechanism of the repression involved, we may assume that there is some universal enzyme U present in the cell which splits, in general, the covalent bond by which the newly formed enzyme molecules are attached to their enzyme forming site. The repressor molecule when it is combined with an attached enzyme molecule may then set up a "steric hindrance" and thus prevent the universal enzyme U from splitting the covalent bond.

When an amino acid, a purin or a pyrimidin, represses the formation of an enzyme which catalyses an early step in the bio-synthetic pathway, then there is, in general, little chemical resemblance between the substrate of the enzyme and the amino acid, the purin or the pyrimidin, which lies at the end of the bio-

synthetic pathway. The substrate has a specific chemical affinity to the catalytic site and the aminoacid, the purine or the pyrimidine which represses the formation of the enzyme has a specific affinity to the controlling site. Accordingly, as far as these enzymes are concerned, the combining specificity of the catalytic site and of the controlling site must be quite different.

In the case of the enzymes, however, which lie towards the end of the biochemical pathway, the substrate of the enzyme is likely to be a chemical analogue of the end product of the pathway and may therefore have specific chemical affinity to the controlling site of the enzyme.

The high rate of enzyme production which may be obtained in the case of boostable enzymes by reducing the intracellular concentration of the metabolite moity of the repressor very low may represent the full rate of enzyme formation of which the ribosome of the enzyme is capable.

On the basis of this model we may expect the following:

1) A chemical analogue $\bar{M}$ of the metabolite moity M may be expected to enhance the formation of the enzyme and thus act as an inducer of the enzyme ~~if the cell is incapable of converting $\bar{M}$ into M.~~

2) In a mutant which is not capable of producing the R moity of the repressor, the enzyme may be produced at a full rate and the enzyme level may be high. ~~Adding the metabolite moity to the growing bacteria culture can not be expected in such a case to repress the formation of the enzyme.~~ Mutants of this kind would be called constitutive.

3) In mutants in which the R moity of the repressor is produced, but is produced ~~at a very low rate so~~ that its rate of production becomes ~~a limited~~ factor for the production of the repressor molecule, we may expect that by adding the metabolite moity of the repressor to a growing culture ~~we may enhance the formation of the enzyme and thus act as an~~ inducer of the enzyme.

Insert on Page 8

The metabolite moity of the repressor 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 moity of the repressor is the substrate itself, the rate of enzyme production should be proportionate to the intracellular concentration of the substrate. The limiting factor for the rate of production of a repressor is the rate of production of the R moity of the repressor.

Generally one may say that the substrate of the enzyme ~~may be within this class of enzymes may be~~ an inducer of the enzyme if with increased intracellular concentration of the substrate, the concentration of the repressor of the enzyme rises more slowly than the concentration of the substrate.

We know empirically that if the enzyme is produced at the "full" rate its amount may represent about 10 % of the total bacterial proteins. Accordingly a concentration $[Z_\infty]$ of such an enzyme may amount to $[Z_\infty] = 10^{-4}$ mol/liter.

3) Degradative enzymes.

The enzymes involved in the degradation of tryptophane to β-keto-adipic acid by pseudomonas-fluorescence may be representative for another large class of enzymes. There are seven enzymes involved in this degradation of tryptophane, and the formation of each of them can be greatly 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. fluorescence. R. Y. Stanier estimated their number at more than fifty and estimated that at least 200 enzymes participate in their degradation. The formation of each of these enzymes may be enhanced by adding its substrate to the growing bacterial culture, and we may therefore refer to these enzymes as inducible enzymes.

We assume that enzymes of this class also have two specific combining sites, the catalytic site and the controlling site. In this case and that the combining specificity of the controlling site is the same as or nearly the same as the combining specificity of the catalytic site so that the substrate of the enzyme has a substantial chemical affinity to both the catalytic site and the controlling site.

We are then able to account for the induction of the enzyme by the substrate on the ground that the substrate may compete with the repressor for the controlling site of the attached enzyme molecule. The substrate must indeed induce the enzyme if the bottleneck in the production of the repressor is not the low rate of production of the R moiety of the repressor or else the low rate of condensation of the R moiety with the metabolite moiety of the repressor, but rather the rate of production of the metabolite moiety of the repressor.

because in that case mixing the same substrate will certainly not reduce the conc. of the repressor.

The Equations:

(1) gives the rate of formation of a "boostable" enzyme in the presence of a repressor and a chemical analogue $\bar{M}$ of the repressor (which competes with the repressor for the controlling site of the "attached" enzyme molecule.

(1) $$\text{rate} = \frac{1}{\frac{\rho}{K^*}\tau_E \rho + \tau_E + \tau(AA)}$$ per ribosome

where ρ is the concentration of the free repressor molecules in the cells, i.e. ~~repressor~~ molecules which are not combined, ~~attached~~ with unattached enzyme molecules contained in ~~the cytoplasm of~~ the cell; K^* is the equilibrium constant for the combination of the repressor with the controlling site of an enzyme molecule ~~which is~~ attached to its "ribosome"; τ_E is the average time needed for the ~~disassociation~~ dissociation of a repressor molecule from the ~~controlling site of an~~ attached enzyme molecule; $\tau(AA)$ is the average time which it takes for the ribosome to assemble the aminoacids, ~~and~~ to join them into the polypeptide chain of enzyme molecule; p is given by 2)

(2) $$p = \frac{1 + \tau_M/\tau_E}{1 + \frac{\tau_M}{\tau_E} + \frac{[\bar{M}]}{K_M}}$$

where $\tau_{\bar{M}}$ is the average time for the ~~disassociation~~ dissociation of ~~a~~ chemical analogue $\bar{M}$ of the metabolite moity M ~~of the repressor~~ from the controlling site of an attached enzyme molecule; $[\bar{M}]$ is ~~a~~ the concentration of the chemical analogue $\bar{M}$ in the cell and K_M is the equilibrium constant for the combination of the chemical analogue $\bar{M}$ with the ~~controlling site of the~~ attached enzyme molecule.

For the sake of simplicity we shall assume

$$\tau_M << \tilde{\tau}_E$$

so that we may write

(3) $$p = \frac{1}{1 + \frac{[\bar{M}]}{K_M}}$$

~~Assuming~~ If we assume $\tau(AA) << \tilde{\tau}_E$

we may write (4) in place of (1)

(4) $$\text{rate} = \frac{1}{\tau_E} \frac{1}{\frac{\rho}{K^*}\rho + 1}$$ per ribosome

Insert 1 on page 7 A

~~In (1)~~ In the denominator of expression (1) the first term ~~xxx~~ represents the average time which the ~~xxxx xxxxxxxxxxxxxxxxxxxx~~ attached enzyme molecule spends combined with the repressor, during which time it cannot be liberated. The second term represents the average time which the ~~attached~~ enzyme molecule spends attached to its ribosome while it is not combined with the repressor, and the third term is the time ~~which~~ taken up by the formation of ~~it takes to form~~ the attached enzyme molecule.

(1) Friday (2)

replacement for p–

the equations:

~~If We can~~ shall ~~neglect the time that it takes for a microsome to assemble the proper sequence of amino acids assemble them which are joined into a polypeptide and fold up~~

~~For the sa Same practical reasons we shall assume postulate that the polypeptide of what which folds up to form an any enzyme~~

We shall ~~assume that~~ designate by τ the average ~~time τ~~ which an ~~any~~ enzyme ~~protein~~ formed ~~molecule~~ ~~that is~~ any molecule remains tied to its "microsome" ~~it~~ (when its detachment from the microsome is not hindered by a repressor molecule having combined with the controlling site).

We shall assume that this time τ is large compared to the time it takes for the polypeptide to form and fold up to form the attached enzyme molekule. In these circumstances ~~the enzy per~~ we may say that there is ~~always an~~ practically an enzyme molekule attached to the microsome, which is either combined with a repressor mole or not.

~~The rate of formation of an~~

New page 7A Frnadas (1)

~~enzyme~~ enzyme ~~- If this rate of is limited only by the repressor present in the cell~~ (1)

If these assumptions hold we may say that the rate of formation of the enzyme is limited only by the amount of a repressor (and only one repressor) present in the cell and we say that the enzyme ~~is of intermediate durable~~ "fully unstable" ~~without limit~~ by repression by one repressor. The rate of formation of such an enzyme per ~~unit~~ ribosome (or microsome) ~~ho~~ is given by

$$\text{rate} = \frac{1}{\tau} q$$

where q is the probability that the attached enzyme is not combined at its controlling site with a ~~r~~ repressor molecule. We may write for q in the presence of a repressor R and an inducer M

$$(1) \quad q = \frac{1}{1 + \quad ,} + \frac{\frac{M}{K^*_M}}{1 + +}$$

or

~~(2)~~ where

In (1) the first term gives the fraction of the att. enzyme molecules which are not combined with either the repressor $R E P_j$ or the inducer M_j and the second term

this holds true if the time that the inducer M takes to dissociate from the controlling site of the att. enz. molecule is short in comparison with

We may write (1) ~~[illegible]~~ Finding (3) also in the form

$$\text{rate} = \frac{1}{\tau} \frac{1 + \frac{[M]}{K_M^*}}{1 + \frac{[M]}{K_M} + \frac{\rho}{K_R}}$$

for zero concentration of the repressor we have $\rho = 0$ and

(3) $$\text{rate}_{max} = \frac{1}{\tau}$$

~~The rest~~ The concentration of an enzyme in a bacterium that grows at a fixed rate is proportional to the rate at which the enzyme is produced, accordingly we may write for the concentration of ~~the~~ any enzyme of which is fully derepressed

(4) $$z = z_0 \frac{1 + \mu}{1 + \mu + \rho}$$

where z_0 is the concentration which the enzyme attains in the cell in the absence of any repressor.

In the absence of ~~the~~ an inducer M we may write

(5) $$z = z_0 \frac{1}{1 + \mu}$$

The Equations:

We shall designate by τ the average time for which a newly formed enzyme molecule remains tied to its enzyme forming site (when its dissociation from its enzyme forming site is not hindered by a repressor molecule that is combined with the controlling site). We shall assume that this time τ is large compared to the time that it takes for the polypeptide to form 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.

We may write (also in the form

For 0 concentration of the repressor we have and we have

The concentration of an enzyme in a bacterium that grows at a fixed rate is proportionate to the rate at which the enzyme is formed. Accordingly we may write for z the concentration of an enzyme which is fully boostable

where z is a concentration which the enzyme would attain in the cell in the absence of any repressor if it were not for the fact that at high enzyme concentrations something other than the concentration of the repressor may limit the rate of formation of the enzyme.

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

The Equations:

(1) gives the rate of formation of a "boostable" enzyme in the presence of a repressor and a chemical analogue $\bar{M}$ of the repressor's metabolytic moity M which competes with the repressor for the controlling site of the "attached" enzyme molecule.

If these assumptions hold, we may then say that the rate of formation of the enzyme is limited only by the amount of a repressor, and only one repressor, present in the cell. In this case we may designate the enzyme as fully boostable. The rate of formation of such an enzyme, per enzyme forming site, is given by

$$\text{rate} = \frac{q}{\tau} \qquad (1)$$

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 q in the presence of a repressor REP and an inducer $\bar{M}$

$$q = \frac{1}{1 + \frac{\mu}{K^*_{\bar{M}}} + \frac{\rho}{K^*_{REP}}} \qquad (2)$$

where μ and ρ are the concentrations of the inducer $\bar{M}$ and the repressor REP respectively, and $K^*_{\bar{M}}$ and K^*_{REP} are the equilibrium constants for the $\bar{M}$ combination and the repressor REP respectively.

In (2) the first term gives a fraction of the attached enzyme molecules which are not combined with either the repressor REP or the inducer $\bar{M}$, and the second term represents a fraction of the attached enzyme molecules which are combined with the inducer $\bar{M}$.

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

We may write ~~(also in the form)~~

$$(3)\qquad \text{rate} = \frac{1}{\tau}\,\frac{1 + \frac{\mu}{K^*_M}}{1 + \frac{\mu}{K^*_M} + \frac{S}{K^*_{REP}}}$$

~~For Ø concentration of the repressor we have~~ and we obtain for the rate

$$\text{rate} = \frac{1}{\tau}\,\frac{1}{1 + \frac{S}{K^*_{REP}}}$$

The concentration of an enzyme in a bacterium that grows at a fixed rate is proportionate to the rate at which the enzyme is formed. Accordingly we may write for z the concentration of ~~an~~ the enzyme ~~which is fully boostable~~

$$(4)\qquad z = \frac{1 + \frac{\mu}{K^*_M}}{1 + \frac{\mu}{K^*_M} + \frac{S}{K^*_{REP}}}\, z_0$$

where z_0 is the concentration which the enzyme ~~could~~ might attain in the cell, in the absence of any repressor ~~if it were not for the fact that at high enzyme concentrations something other than the concentration of the repressor may limit the rate of formation of the enzyme.~~

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

$$(5)\qquad z = \frac{1}{1 + \frac{S}{K^*_{REP}}}\, z_0$$

and for low repressor concentrations where we have

$$\frac{\rho}{k} \ll 1$$

we obtain from (4)

$$(5) \qquad \text{rate} = \frac{1}{\tau_E}$$

per ribosome

for the corresponding enzyme concentration z we may write on the basis of (4)

$$(6) \qquad z = \frac{c_0}{\tau_E \rho/k + 1}$$

where c_0 is a constant.

and for we write from (5) we write for z_∞, the concentration of the enzyme in the fully boosted cell.

$$z_\infty = \frac{c_0}{\tau_E}$$

(7) and we may also write

(8) $\frac{z}{z_\infty} = \frac{1}{k\rho + 1}$

We may compute ρ the concentration of the free repressor molecules from the concentration ρ_0, the total concentration of the repressor molecules (both free and bound) by writing

$$(9) \qquad \rho = \rho_0 - z \frac{\rho/k}{1 + \rho/k}$$

(unattached enz molecules)

where K designates the equilibrium constant for the combination of the repressor molecule with the "unattached" enzyme molecules present in the cell. In this expression (9) the first term represents the total concentration of the repressor xxxxxxxx in the cell, and the second term represents the concentration of those repressor molecules which are combined with an unattached enzyme molecule. In a growing bacterial culture the total repressor concentration ρ_0 which establishes itself in the stationary state which xxxxxx is determined by the proportional constant rate at which the repressor molecules are produced and the constant rate concentrate of growth of bacteria.

Insert II

From (3) and (6) it is described by

On the basis of the model and the above given equations which describe the behavior of the model, we may expect the following:

1) If the cells of a bacterial strain are incapable of converting a close chemical analogue $\bar{M}$ of the metabolite moity M into this metabolite moity, then (in general

of the repressor

the chemical analogue ~~will~~ enhance the formation of the enzyme if it is added to growing bacterial culture and ~~provided it can get into~~ the bacterial cells. Accordingly the chemical analogue M ~~is~~ an inducer of the enzyme in such a bacterial strain.

2) ~~In a~~ mutants which ~~is~~ not capable of producing the R moity of the repressor, the enzyme ~~may~~ be produced at the full rate and the enzyme level ~~may~~ be very high. In such a case ~~we may not expect to be able~~ to repress the formation of the enzyme by adding the metabolite moity of the repressor to the bacterial culture. Mutants of this ~~kind~~ ~~will be designated as~~ "constitutive" ~~mutants~~.

3) There may exist mutants in which the R moity of the repressor is produced, but is produced ~~in~~ at such a low rate that its production may be ~~come~~ the limiting factor for the production of the repressor ~~if~~ the intracellular concentration of the metabolite moity of the repressor is ~~reasonably high~~. ~~We may expect that~~ in such a mutant the formation of the enzyme ~~can~~ be enhanced by adding the metabolite moity ~~of the repressor~~ to the growing bacterial culture. Accordingly for such a mutant the metabolite moity of the repressor will be an inducer of the enzyme.

4) 4.) In a mutant in which the R moity of the repressor is produced at a very low rate the enzyme will be maintained in the cell - in the absence of an inducer - at a level which is substantially above the enzyme level of an uninduced wild type strain, but still substantially below the enzyme level which attained in the wild type strain, in the presence of the inducer. In such a mutant the reduction of the free repressor concentration by the presence of the enzyme might manifest itself in the kinetics of the formation of the enzyme. If the mutant is grown in the absence of the inducer and then at a given point in time, the inducer level within the cell is raised to a moderately high level, the enzyme content of the cell will rise and along with the enzyme content of the cell should also rise the rate of formation of the enzyme.

Such a phenomenon may be expected on the following basis:

The total concentration of the repressor molecules in a growing bacterium is determined by the rate at which the repressor is produced, and the rate at which the repressor is diluted by the rate of the increase in the total bacterium volume of the growing culture. The rate of enzyme production, however, is determined not by the total amount of repressor present in the cell but rather by the fraction which is uncombined. Certain fractions of the repressor molecules will be combined with the controlling site of the unattached enzyme molecules present in the cell and therefore the more enzyme is present in the cell the lower will be the concentration of the free repressor molecules. Hence the rate of enzyme production must, at least in principle, rise with the amount of the enzyme present in the cell.

whether this effect is appreciable or not depends on the values of the constants which control the behavior of the equations quoted above.

5) If the constants controlling these equations fall within certain ranges we might have -- at present hypothetical -- a bacterial strain which may be called para-constitutive. In such a strain the enzyme level might be quite low, but if once the enzyme level is raised from then on there will be sustained a higher rate of production of the enzyme -- in the absence of an external, or even internal, inducer. And when the cell divides, the daughter cells also maintain enzyme level, so that we are dealing here with a hereditary property of the cell even though the inheritance is not genetic. This behavior of the cell is due to the fact that there is no inherent instability within certain ranges of the constants controlling the equations. No such strain has so far been found. Because there is so far no experimental evidence for such para-constitutive bacterial strains we will not enter into a detailed discussion of this hypothetical phenomenon here.

The inherent instability here mentioned may, however, form the molecular basis of cellular differentiation. And it may form the molecular basis of sustained antibody production by mammals that may follow the injection of an antigen for which the antibody is specific. In the case of differentiation the initial raising of the enzyme level to a point where high rate of enzyme formation becomes self-sustaining may conceivably occur through the lowering of the production of the R moiety of the repressor which is specific for the enzyme. In the case of antibody production the initial raising of the antibody level, which results from the injection of the antigen for which the antibody is specific, may be brought about by reduction in the production rate of the metabolite moiety of the repressor which controls the rate of formation of the antibody that is specific for the antigen injected.

These phenomena will be discussed in greater detail in a second paper contained in the present issue.

Insert on page 7 E

5.) If for ~~such~~ a ~~strain~~ we have $\rho_0 \ll Z_0$, and if the other constants controlling ~~the~~ equations ~~(4)~~ fall within a certain range, we may be dealing with a mutant which ~~we might call~~ "para-constitutive". ~~The~~ enzyme ~~level~~ in such a para-constitutive strain, need not be very high, but if once the enzyme ~~xxxxxxxxxxxxxxx~~ concentration is raised to a high level, then from thereon a higher rate of production of the enzyme ~~will~~ be permanently sustained - even in the absence of an external or internal inducer. When such a cell which sustains a high enzyme level, divides, the daughter cells will also sustain a high enzyme level so that ~~xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx~~ sustaining a high enzyme level is a hereditary ~~property of the cell~~, even though the inheritance is not genic. No "para-constitutive" mutants have so far been found in bacteria, and therefore they ~~xxxx~~ represent so far a figment of the imagination. We have conjured up here ~~this figment of imagination~~ as a means of stressing the fact that the enzyme forming system described by our equations is inherently instable, within certain ranges of the constants which control these equations.

Equations for the enzyme forming system in mammalian cells.

We shall now examine under what conditions the cell may sustain at a high rate the formation of an enzyme which is normally produced by the cell at a low rate.

We may write for z, the concentration of the enzyme in the cell at which its rate of formation is self sustaining from (10) and (11).

(10) $$\rho = \rho_0 - z\frac{\rho/k}{1+\rho/k}$$

or (10A) (2 lines)

and

(11) $$z = \frac{z_0}{1+\rho/k}$$

or (11A) (2 lines)

In (10) the first term represents the total concentration of the repressor in the cell and the second term represents the concentration of the repressor molecules in the cell which are bound to the controlling site of the "unattached" enzyme molecules which are present in the cytoplasm of the cell.

From (10) we obtain

(12) $$\frac{\rho}{k} = \frac{+\frac{\rho+\rho_0+z-k}{k} + \sqrt{\left(\frac{z-\rho_0+k}{k}\right)^2 + \frac{4\rho_0}{k}}}{2}$$

We shall henceforth assume that we have (13)

(13) $$z_0 >> 2\rho_0$$

~~and~~

~~(14) $\rho_0 >> 4k$~~

~~Within the range of constants for which the inequalities (13) and (14) hold,~~ the equations (10) and (11) may have two stable solutions. For one of these, z, the concentration of the enzyme in the cell is high, and for the other it is low.

For the solution which corresponds to the higher enzyme concentration, we may assume

(13) $$z >> 2\rho_0$$

In this case we also have

4 (15) $$(z+k)^2 >> 4\rho_0 k$$

and accordingly we may write from (12)

(15) (~~17~~) (~~16~~)
$$\frac{\rho}{K} \approx \frac{\rho_0}{z+K}$$

We may write (11) in the form

(~~18~~) (13)
$$\frac{\rho}{K^*} = \frac{1 - z/z_0}{z/z_0}$$

From (~~17~~ 15) and (~~18~~ 17) we obtain

(~~16~~)
$$\left(\frac{z}{z_0}\right)^2 + \left\{\frac{K}{z_0}(\lambda+1) - 1\right\}\frac{z}{z_0} - \frac{K}{z_0} = 0$$

where λ stands for $\lambda = \frac{\rho_0}{K^*}$

From (~~19~~ 18) we obtain

(~~20~~)
(17) (~~19~~) (~~20~~)
$$\frac{z}{z_0} = \frac{1 - \frac{K}{z_0}(\lambda+1) \pm \sqrt{\left\{1 - \frac{K}{z_0}(\lambda+1)\right\}^2 + \frac{4K}{z_0}}}{2}$$

If we have

18 (~~20~~) (~~21~~)
$$\left(1 - \frac{K(\lambda+1)}{z_0}\right)^2 >> \frac{4K}{z_0}$$

then we may write from (~~19~~ 17)

(~~22~~)
(19)
$$\frac{z}{z_0} \approx 1 - \frac{K(\lambda+1)}{z_0}$$

or

(19A)
$$\frac{K(\lambda+1)}{z_0} \approx 1 - \frac{z}{z_0}$$

(26)

p. 10

If $\lambda = 10^7$ 1% of proteins do 1 job

$z_0 = 10^{-3}$

non lock $K > 10^{-10}$

~~to get lock K has to fall to~~

assume $K = 10^{-5}$ to get lock $\lambda =$

$10^{-5} \lambda = 10^{-3}$

$\lambda = 10^2$

or if 10 identical enzymes (unbranched)

$\lambda \equiv 10^3$ will give lock

ρ_0 must be small

for $\lambda = 10^2$ $K^* = 10^{-13}$

$\rho_0 = 10^{-11}$

$\lambda = 10^7$ $\rho_0 = 10^{-6}$