August 30, 1957

On the Formation of Adaptive Enzymes

by Leo Szilard
The Enrico Fermi Institute for Nuclear Studies
The University of Chicago, Chicago, Illinois

It would appear that as a general rule -- with notable exceptions -- bacterial cells respond with the formation of adaptive enzyme to a great variety of compounds that they can metabolize, and many of these compounds resemble some degradation product of an amino acid or a nucleic acid residue. How does the bacterial cell accomplish this feat?

Largely due to the work of Jacques Monod and his co-workers in Paris, and more recently also through the work of Aaron Novick and Milton Weiner in Chicago, much information has become available on the induced formation of the enzyme, β-galactosidase.

This enzyme splits the disaccharide lactose into glucose and galactose, and if the bacteria are grown in the presence of a high concentration of a chemical analogue of lactose, the β-galactoside TMG, which is not split by the enzyme, the enzyme level may be raised in the growing culture from a few molecules per bacterium, depending on the concentration of TMG used, up to perhaps 10,000 molecules of this enzyme per cell. When a bacterium is used that requires for its growth an amino acid -- tryptophan for instance -- and when such a bacterium is grown in the Chemostat, using tryptophan as a controlling growth factor, then the generation time, τgen, can be stretched, at will, up to perhaps tenfold. It is known that the enzyme level reached, when the bacteria are grown, at a fixed concentration of TMG, in this manner in the Chemostat is independent of the generation time, τgen. This, of course, would be expected at very high TMG

concentrations when the bacteria are fully induced, but it is also true for lower inducer concentrations when the enzyme level is appreciable but not at its maximum possible value. This apparent "law of the growth rate independence of the enzyme level" furnishes, to my mind, an important clue to the general mechanism of enzyme induction. Moreover on the basis of this mechanism, one should expect a more general law to hold, which says that the enzymatic composition of the bacterium does not change when we reduce the growth rate -- over a range of perhaps a factor of ten -- by slowing the rate of protein synthesis. Our formulae indicate the possibility of an exception to this rule in the case of mutants which contain an abnormally high level of an inducible enzyme in the absence of an inducer -- the so-called constitutive mutants. Perhaps one has to regard such mutants as pathological organisms, in which the normal regulation of the level of an enzyme has broken down.

How can an inducer raise the enzyme level from a few molecules per cell to about 10,000 molecules per cell? According to the notions here adopted, the enzyme is formed from amino acids which line up alongside a nucleic acid strand and then are joined by some chemical reaction chain into a polypeptide that, in turn, folds up into an enzyme. The nucleic acid strand, which imposes a specific amino acid sequence on the polypeptide must contain the same information as does the gene but need not necessarily be the gene itself, and therefore we shall refer to it as the "paragene."

For the sake of argument, I shall assume in this presentation that the uninduced cell contains one paragene for each kind of enzyme that the cell is capable of producing. If the enzyme level is raised several thousandfold, through the action of an inducer added to the medium, we can

then either say that the inducer somehow increases the number of paragenes which are specific for this enzyme -- a possibility which we shall disregard -- or else we can say that the rate at which the enzyme is made by this paragene is increased by the inducer and that no additional paragenes of this specificity are produced.

If a paragene is fully induced, the rate of enzyme production is determined by the time, $\tau(AA)$, that it takes to assemble all the required amino acids along the paragene and the time, $\tau(E)$, that it takes for the enzyme to evaporate from the paragene after it has been formed. Accordingly in the fully induced cell the number of specific enzyme molecules present in the cell is given by

$$N_{max} = \frac{\tau_{gen}}{\tau(E) + \tau(AA)} \qquad (1)$$

As we shall see later, the mechanism proposed will give results which are in accordance with the observed experimental facts only if we assume

$$\tau(E) \ll \tau(AA) \qquad (2)$$

In the case of full induction of the enzyme, β-galactosidase, in bacteria growing at a fast rate (τ_{gen} = 5,000 seconds) in the Chemostat, one finds $N_{max} \sim$ 10,000 molecules per cell, and accordingly one obtains from equations (1) and (2)

$$\tau_{min}(AA) \approx \frac{1}{2}\ \text{sec} \qquad (3)$$

It is believed that there might be about 10,000 different genes in a bacterium which contains about 10^{-13} grams of protein. If, on the average, the corresponding 10,000 different enzymes have a molecular weight of 100,000, then there must be on the average of each kind of enzyme 60 molecules present in the cell. The notion here adopted is that every paragene could maintain about 10,000 molecules per cell in a growing bacterial culture, if it produced the enzyme at the full rate, and that on the average the rate of enzyme production of the paragenes is repressed by a factor of 200. What represses the rate at which a paragene produces an enzyme?

One might attempt to account for the phenomenon of induction by saying that perhaps the enzyme formed along the paragene sticks to the paragene until a hypothetical inducer molecule combines with the paragene-enzyme complex, and thereby somehow releases the enzyme from the paragene. The paragene-enzyme complex -- so one would then say -- cannot synthetize further enzyme molecules until the enzyme leaves the paragene. It is easy to show that such a model would be in flagrant contradiction to the law of growth-rate independence on the level of the externally induced enzyme. This model demands that, if there are maintained, say, 1,000 enzyme molecules per cell when the bacteria grow in the Chemostat at a certain growth rate and at a certain inducer concentration, the enzyme level ought to drop by a factor of ____ when the growth of the bacteria is slowed by a factor of 2 through the lowering of the level of the

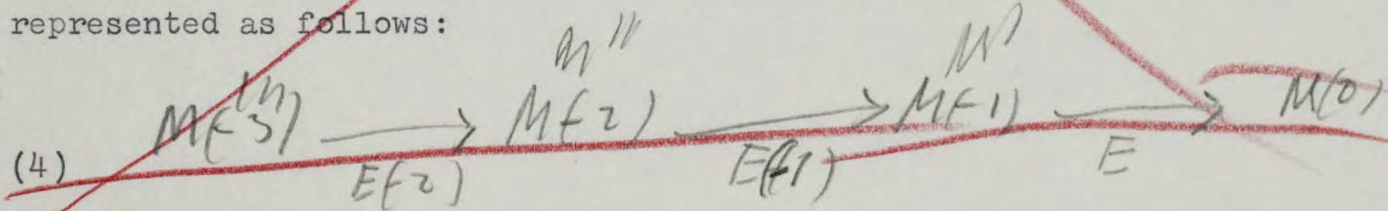
Replacement for page 5

We might attempt to account for the phenomenon of enzyme induction by saying that perhaps the enzyme formed along the paragene sticks to the paragene and thereby prevents the formation of additional enzyme molecules until a hypothetical inducer molecule combines with the enzyme-paragene complex and thereby somehow releases the enzyme from the paragene. It is, however, possible to show that such a model could not account for the ""law of growth-rate independence of the level of an induced enzyme."

Let us now consider two bacterial cultures, each of which is growing at the same inducer concentration in a Chemostat with, say, tryptophane as a controlling growth factor, and one of them growing twice as fast as the other. According to the model just presented, if the enzyme is, say, a few thousand molecules per bacterium (but still sufficiently below N_{max} = 10,000), then the enzyme level in the slow growing culture would be very much lower, perhaps about ________ enzyme molecules per bacterium. All experience contradicts such an expectation. We shall, therefore, attempt here to present a different model for enzyme induction which might incidentally account not only for enzyme induction but also for the phenomenon of enzyme repression first described by Vogel. Vogel found that the enzyme, acetyl ornithase, one of the enzymes in the biochemical pathway leading to arginine, is absent when the bacteria grow with a high concentration of arginine in the nutrient.

For the purposes of the immediately following discussion, we may assume that we are dealing with an enzyme that lies in the biosynthetic pathway leading to an amino acid. Such a biosynthetic pathway may be symbolically represented as follows:

(4)



Moreover it would seem reasonable to demand that the correct model for enzyme induction should explain also the phenomenon of enzyme repression and perhaps even the phenomenon of antibody formation in mammalian organisms. The phenomenon of enzyme repression was first observed by Vogel.

Vogel found that the enzyme, acetyl ornithase, one of the enzymes in the biochemical pathway leading to arginine, is absent when his bacteria grow at a high concentration of arginine in the nutrient.

For the purposes of the immediately following discussion, we may assume that we are dealing with an enzyme that lies in the biosynthetic pathway leading to an amino acid. Such a biosynthetic pathway may be symbolically represented as follows:

$$(4)\quad M(-3) \xrightarrow{E(-2)} M(-2) \xrightarrow{E(-1)} M(-1) \xrightarrow{E(0)} M(0)$$

The metabolite, M(0), in this formula may be taken to be any amino acid and the metabolites, M(-3), M(-2) and M(-1) represent precursors of this amino acid. If the amino acid, M(0), is arginine, these precursors are in order acetyl ornithine, ornithine, and citrulline. The enzyme, E(-2), is acetyl ornithine.

We shall now attempt to present here a model for enzyme induction which will account also for enzyme repression and which might further account for the phenomenon of antibody formation in mammalian organisms. The reader might, for fixing his mind, assume to begin with that we are dealing with the production of an enzyme that lies on the biosynthetic pathway leading to an amino acid. Later on it will be possible to consider other cases also. The model here presented assumes that amino acids are assembled in a specific sequence, along a specific paragene, and are joined into polypeptides through

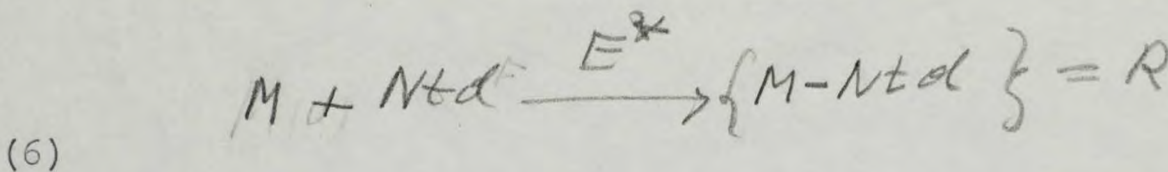
some chemical reaction chain. This polypeptide, in turn, folds up into the enzyme. On the average this process takes a time, τ(AA). If we deal with a bacterium that requires, for instance, tryptophan, as a growth factor, the generation time, τ(AA), can be stretched at will up to perhaps tenfold by growing the bacteria in a Chemostat and lowering the concentration in the growth tube in the usual manner. At high concentration in the medium, we obtain the minimal value for τ(AA), and we have

τ(AA) ≈ 5000 sec

According to the notions here presented, there are present in the cell metabolites, which we shall call repressor molecules and which — we shall describe presently in greater detail which can combine with the paragene-enzyme complex in a reversible manner, and as long as the paragene-enzyme complex is thus covered by the repressor, the enzyme cannot evaporate. In the absence of a specific repressor or in the presence of a metabolite, M*, that can compete with the specific repressor for the enzyme which sits on the paragene in sufficient concentration, the enzyme formed on the paragene will evaporate off the paragene at the rate, of $1/\tau$(E). Any metabolite, M*, that is a chemical analogue of the metabolite, M, which is produced by the enzyme or the immediate precursor of this metabolite, M, will thus protect the paragene-enzyme complex from being covered by the repressor, and such analogue, M*, will therefore act as a direct inducer, provided only that the cell cannot make a repressor out of it. In contrast to M*, we assume that the metabolite, M, can be converted by the cell into a repressor. The metabolite, M, also protects,

What kind of molecule must the repressor molecule R be in order to be able to prevent the evaporation of the enzyme molecule which has been formed from the paragene? We assume that such a repressor molecule contains a moiety that can combine with the enzyme, such as a metabolite, M(0), and it must contain a moiety, Ntd, which can combine with a certain part of the paragene -- perhaps a group of nucleotides on the paragene. It is conceivable that the moiety, Ntd, might be composed of nucleotides; hence the designation, Ntd. For such a molecule, R, to function as a repressor, it is necessary that it "fit" the paragene-enzyme complex in the sense that the moiety, M, must be able to hold on to the enzyme at the same time as the moiety, Ntd, holds on to the paragene. This imposes many specific conditions on the spatial configurations of the repressor, R.

Such a repressor molecule, R, may be formed in the cell from the metabolite, M, by the reaction



$$M + Ntd \xrightarrow{E^*} \{M\text{-}Ntd\} = R \qquad (6)$$

The enzyme, E*, may be called "coupling enzyme" and such a coupling enzyme, as well as the moiety, Ntd, might be specific for each metabolite, M, although a number of metabolites along the same biochemical pathway might be able to react with the same coupling enzyme and the same Ntd moiety.

A chemical compound, M*, that is a chemical analogue of a metabolite, M -- (which is metabolized by the enzyme E) (or its immediate precursor M') and that can combine with the

We shall now define as a real inducer (in contradistinction from quasi inducers to be defined later.)

enzyme molecule that has been formed and sits on the paragene and that the cell is unable to couple to/Ntd moiety to form a repressor, will enhance the formation of the enzyme and will be called an inducer. It will do so by reversibly combining with the enzyme moiety of the paragene-enzyme complex and during the time when the enzyme is occupied by the inducer, M*, the paragene-enzyme complex is protected from being covered by a repressor molecule. The enzyme which is thus combined with the inducer, M*, evaporates off the paragene at the same rate, $1/\tau(E)$, as the enzyme which is uncombined with anything.

If the enzyme which sits on the paragene is combined with the metabolite, M, it will also evaporate at the same rate, $1/\tau(E)$, and thus the metabolite, M, also protects the paragene-enzyme complex against being covered by the repressor. However, this effect of the metabolite, M, may or may not be counterbalanced by virtue of the fact that the cell can transform the metabolite, M, into a repressor, and the metabolite, M, may therefore raise the concentration of the repressor molecules in the cell. Accordingly, the metabolite, M, will, depending on the circumstances, either enhance the formation of the enzyme or repress it, in contradistinction to the chemical analogue, M*, which cannot be transformed into a repressor molecule and must therefore always enhance enzyme production.

There is another difference beween M and M*. Where M as well as M* may enhance enzyme formation directly by combining with the enzyme sitting on the paragene and thereby protecting the paragene enzyme complex from being covered by the repressor, M* can in addition enhance the formation of the enzyme indirectly. It can do this in two ways. M* by competing with the metabolite M(n) for the enzyme, E(n+1) which transforms the metabolite, M(n)

into the metabolite, M(n+1), may enhance the formation of the enzyme, E(n+1), both by raising the concentration of the metabolite, M(n) and by decreasing the concentration of the metabolite, M(n+1), from which the cell may form the repressor, R(n+1).

The combination of the metabolite, M, and the inducer, M*, with the enzyme is reversible and so is the combination of the repressor, R, with the paragene-enzyme complex.

The model here adopted assumes that when the repressor evaporates from the paragene-enzyme complex it leaves the enzyme on the paragene.

Having thus described qualitatively a model for enzyme induction and repression, we shall now proceed to compute the number of enzyme molecules per cell which may be maintained in a growing culture, depending on the concentrations of M, M*, and R which are maintained in the cell. The concentration of M, M*, and R, that are maintained in the cell, we shall designate by [M], [M*], and [R]. For the Michaelis constants of these compounds, we may write K(M) and K(M*) respectively. Similarly we may write for the average time which it takes for M and M* to evaporate off the enzyme and for R to evaporate off the paragene-enzyme complex, $\tau(M)$, $\tau(M^*)$, and $\tau(R)$, respectively.

In order to be able to give a formula for the number of molecules of a given enzyme maintained per cell in the growing culture, it is necessary to make certain assumptions concerning the evaporation times, $\tau(M)$, $\tau(M^*)$, $\tau(R)$ and $\tau(E)$. Our assumptions will be as follows:

(7) $\tau(R) >> \tau(E)$

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

(9) $\tau(M^*) << \tau(E)$

(8) $\tau(M), \tau(M'), \tau(M^*) >>$

new

For reasons that will be stated later, we shall also assume

(9) $$\tau(E) << \tau(AA)$$

The times $\tau(M)$, $\tau(M^*)$ and $\tau(R)$ may be roughly estimated by writing

(10) $$\frac{1}{\tau} = 10^{13} e^{\frac{-\Delta H}{RT}};$$

where $\Delta H(M)$, $\Delta H(M^*)$ and $\Delta H(R)$ represent the binding energy of these compounds to the enzyme or enzyme-template complex, and by making a rough estimate of these binding energies we may surmise that we have $\tau(M) < \frac{1}{100}$ sec; $\tau(M') < \frac{1}{100}$ sec; $\tau(M^*) < \frac{1}{100}$ sec; $\tau(R) >> 1$ sec

For $\tau(AA)$, we obtain from a rather rough considerations $\tau(AA) \sim 1$ second.

We have no way of estimating directly $\tau(E)$ but since, for reasons to be stated later, we are forced to assume that $\tau(E) << \tau(AA)$, we may hazard as a rough guess that we might perhaps have $\tau(E) \sim \frac{1}{10}$ sec

Assuming that inequalities 7, 8 and 9 hold we obtain for N_1 the number of molecules of a given enzyme that is maintained in the cell in the growing culture

(12) $$N = \frac{\tau_{gen}}{\tau(E)\left(1 + \frac{[R]}{K(R)}\,p\right) + \tau(AA)}$$

where

(13) $$p = \frac{1}{1 + \frac{M}{K(M)} + \frac{M'}{K(M')} + \frac{M^*}{K(M^*)}}$$

new

For high values of M*; i.e. if the cell is fully induced, we obtain from equation (10) N_{max}

$$(12)\qquad N_{max} = \frac{\tau_{gen}}{\tau(E) + \tau(AA)}$$

As we have mentioned before, in a fully induced cell there are about 10,000 molecules of the enzyme; i.e. we may put N_{max} = 10,000. Accordingly, for a cell which is growing in minimal medium, supplemented with tryptophan, at a fast rate, so that we have $\tau(gen)_{min}$ = about 5,000, we obtain from (12) $\tau(E) + \tau(AA)$ = 1/2 sec., and since we are assuming $\tau(E) < \tau(AA)$, we obtain $\tau(AA)_{min}$ = 1/2 sec. STOP In trying to compare our equation (10) with experience, we will have to discuss separately ~~the behavior of enzymes along~~ metabolic pathways which lead to an amino acid. ~~In these cases it has so far never been found that an intermediate~~ metabolite enhances the level of any of these enzymes. But the enzyme level can be raised by lowering the concentration of an amino acid in the cell below the level that is maintained when the wild type is freely growing in minimal medium. There are ~~also known~~ cases where adding the amino acid to the nutrient medium represses an enzyme lying along the biochemical pathway that ~~kies~~ leads to that amino acid, and there are ~~other~~ cases where ~~adding the amino acid to the medium does not repress the level of such an enzyme.~~

~~After examining what predictions we may make on the basis of equation (10) for this class of enzymes, we shall then turn to a different class of enzymes.~~

[handwritten annotations in red, partly illegible: "now four different groups of enzymes lying along which ... In this group no intermediate is known to ..."; "it does not. ... It ought to be possible — according to our theory to ..."; "find real inducer M* but this has so far not been explored."]

There is a vast area of chemical compounds which certain bacteria can metabolise and with enzymes that appear to be quasi-inducible in our meaning of the term, inasmuch as the enzyme which carries these compounds through a biochemical step is enhanced when the compound is added to the medium. In this class belong, for instance, the enzymes lying on the biochemical pathway which leads step by step from an amino acid through the successive degradation products. Thus tryptophan is degraded in bacteria

Why do we observe an enhancement of the enzyme by a metabolite in this class of enzyme when the phenomenon must be quite rate with enzymes that lie on the biochemical pathways that lead to an amino acid?

I believe that the difference between these two classes is the following: In the case of the first group the concentration of the repressor is primarily determined by the concentration of the metabolite that can be converted to the repressor because neither the coupling enzymes nor the Ntd moieties are the limiting factor. We may therefore write

(13) $$[R] = C\,[M]$$

In contrast to this, in the second group of metabolites and enzymes, the concentration of the repressor is determined either by the coupling enzymes present or by the supply of the Ntd moieties, and therefore when we raise the concentration of the metabolite, the concentration of the repressor remains unchanged at some level $R = R(0)$.

(14) $$\frac{d[R]}{d[M]} \cong 0$$

We shall now first of all examine what predictions we can make on the basis of equation (10) if we assume for the first group of metabolites and enzymes that equation (13) holds.

4th Version May 13, 1957

Control of Enzyme Production, Suppressor Genes and Enzyme Induction in Microorganisms, as well as Drug Tolerance and Antibody Formation in Mammals

By Leo Szilard

The Enrico Fermi Institute for Nuclear Studies
The University of Chicago, Chicago, Illinois

Introduction

I shall attempt to present in this paper the groundwork for a quantitative theory that covers a wide range of biological phenomena, and includes enzyme induction in microorganisms as well as antibody formation in mammals. From the point of view of the notions here presented, enzymes may not be classified as inducible or constitutive, as it was customary to do hitherto. The real distinction is between two - quite differently circumscribed - classes of enaymes: To one class belong the enzymes that lie along the biosynthetic pathways which lead to an amino acid, purine or pyrimidine; i.e. to a building block of the essential polymers of the cell, such as the proteins and nucleic acids. Enzymes belong to this class are - as a general rule - not manifestly inducible. Yet many, if not all, of them are inducible in principle and some of them may actually be inducible under experimental conditions.

To the other class belong the enzymes involved in the biosynthetic pathways along which the above mentioned building blocks are step by step degraded or otherwise modified. As a general rule an enzyme belonging to this second class may be inducible by the precursor of the metabolite which it produces. The main emphasis of this paper will be laid on this class of enzymes.

It is presumed that enzymes are each produced by a specific template, and according to the concepts developed in this paper, each such template is potentially capable of producing a corresponding enzyme at about the same rate as a cell is able to make all of its proteins. This rate is probably limited only by the rate at which the amino acids are supplied (which are the building blocks of the proteins). The reason that the various templates produce enzyme at a much lower rate is due to the fact that the enzyme-template-complex is reversibly combined with a specific repressor, and the enzyme-template-complex which is thus "covered" does not dissociate off enzyme.

According to the notions here developed, the production rate of most specific enzymes is thus repressed by a factor which lies somewhere between 100 and 10,000, and which is quite different for each specific enzyme.

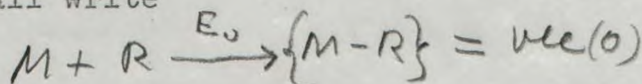
What are these compounds which act as repressors?

Let us, as an example, consider the enzymes which catalyze the last biosynthetic step that leads to a fundamental metabolite, M, such as an amino acid, purine or pyrimadine (which are the building blocks from which the proteins, enzymes and the various ribonucleic acids and desoxyribonucleic acid).

For the sake of easier communication, we shall single out for the moment the amino acids. Each amino acid, M, is -- according to this theory -- coupled by a coupling enzyme, E_o (or a system of such enzymes) to some radical, R, to form an intermediate metabolite, {M-R}. We shall refer to this metabolite, {M-R}, as a "vector" because it might be the vehicle (or as a precursor of the vehicle) which transmits the amino acid to the templates that guide the formation of the specific bacterial proteins.

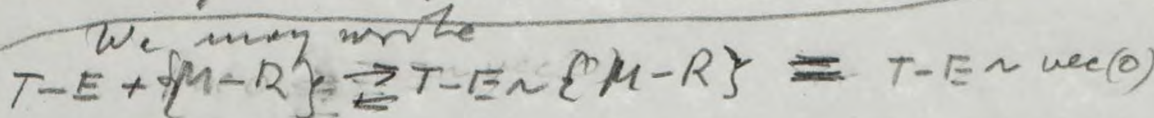
The important role which repressors play in the theory here presented yields, as a by-product, several mechanisms through which a substrate of an enzyme or a chemical analogue of such a substrate may enhance the rate of production of the enzyme; i.e. may act as an inducer of that enzyme. But the problem of enzyme induction goes, as we shall see, far deeper than the question of the mechanism of enzyme induction.

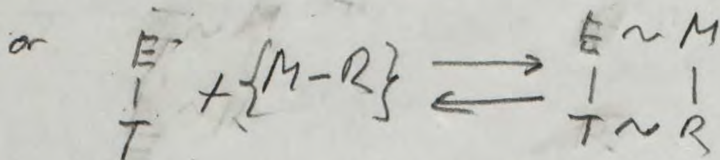
We shall write

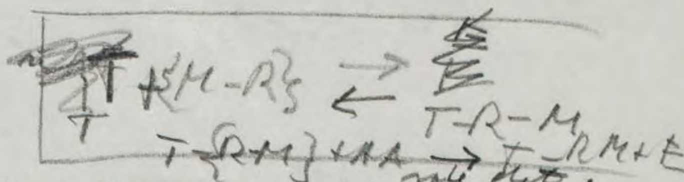


the equilibrium, K(M) constant for the reversible reaction between the template enzyme complex T-E and vec 0

We may write







as well as the concentration of vec(0) determines the rate at which the enzyme is formed that catalyzes the last biosynthetic step which leads to the formation of the amino acid, M. For a given concentration of vec(0) if this equilibrium constant, K(M), is low (i.e. if the binding energy of vec(0) to the enzyme-template-complex is high) the rate of formation of the enzyme will be highly repressed. Occasionally a mutation can occur (in a bacterium) which enhances the equilibrium constant, K(M), from a very low value to a very high value. In that case an enzyme which was present only in a low amount in the wild type bacterium will be present in the mutant in an amount which might exceed by a factor of 10,000 the amount of enzyme present in the wild type. An example for this might very well be the regarded as ... constitutive ... Escherichia coli. The wild type contains only a very small amount of this enzyme. When grown in the presence of an inducer, such as the β-galactoside, TMG, enzyme production is enhanced by a factor of several thousands. Mutants of this strain, however, which are called constitutive have the enzyme present in an amount which is perhaps 10^4 times as great as the amount present in the wild type also when grown in the absence of an inducer. According to our theory the extraordinarily high enzyme level of these mutants can be explained by assuming that the mutation increased the value of the equilibrium constant K(M) by a factor of about 10,000.

No acceptable theory has been proposed so far that would describe a mechanism through which an inducer can enhance the rate of production of an enzyme, but the problem with which we are faced goes deeper than the question of the mechanism of enzyme induction. There is a vast array of compounds which are potentially oxidizable by bacterium, P fluorescence. R. Y. Stanier(1) estimates the number of such compounds at 50, and believes that more than 250 enzymes must participate in their dissimilation. Why are all these enzymes present in a bacterium? Even if there has been some use for them at some time during the long period of their evolution, why would they persist - as I am convinced they would - if we were to cultivate the bacteria for a long period of time in the absence of any of the substrates of any of these enzymes? Why would these enzymes not be gradually lost by mutations during such a period of cultivation while there is no selection operating in their favor? I believe these questions can be answered satisfactorily and that we had better now abandon the belief - repeatedly voiced by previous authors - that "these enzymatic potentialities are stored away in the genome in order to be simply and rapidly activated through sequential induction when a suitable substrate enters the environment." I shall in the concluding chapter of this paper attempt to make it plausible that these enzymes are produced by suppressor genes. Mutations occur during evolution which affect the regulatory mechanism of the cell, and these suppressor genes have been selected because they restore the proper operation of the regulatory system. These genes have become part of the regulatory system that determine the rate of production of the enzymes, and they persist because selection would operate against the bacterium that may lose such a gene through a mutation.

perhaps as many as 1000

~~We shall, for the purpose of this paper, assume that there~~ there might be a 1000 such suppressor genes present, each producing a specific enzyme. These enzymes might be ~~and that these enzymes are present~~ at a very low concentration when the bacteria grow in minimal medium.

~~Support for the view~~ The view that such suppressor genes and their corresponding enzymes are present in microorganisms is supported ~~can be derived from~~ by the experiments of Yanowsky and coworkers in Neurospora. They ~~found~~ have shown that mutations ~~in the Neurospora~~, which lower the rate of production of the enzyme tryptophane synthetase, can be restored by suppressor genes and that the different such mutations, which all lie in the same functional ~~chain~~ gene forming tryptophane synthetase, require the presence of different suppressor genes for restoring the ability of the cell to form the enzyme in adequate amounts. They further found that all those ~~(tryptophane-requiring)~~ mutants for which a suppressor can be found also produce an immunological analogue of tryptophane synthetase. All this is in line with the general notions to which we are led by our theory.

~~The theory here presented~~ This theory establishes ~~the~~ connection between the specificity of the suppressor genes found by Yanowsky and his coworkers and the ability of bacterium like P fluorescence to dissimilate a vast array of oxidizible compounds in a most natural fashion with the help of inducible enzymes, and this then leads to a ~~once this is done we shall be able to present the~~ theory of drug tolerance, withdrawal symptoms and antibody formation in mammals.

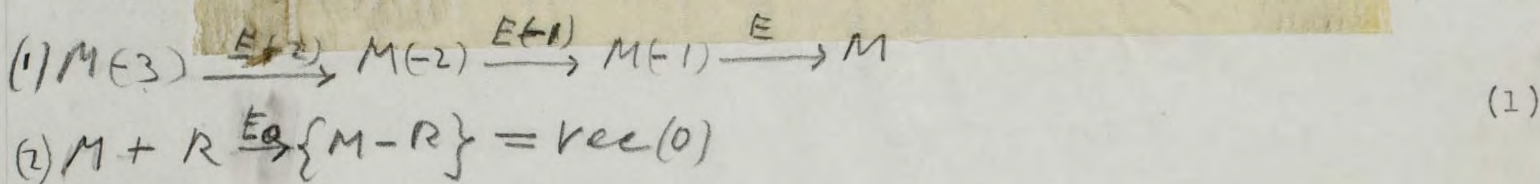
The Basic Scheme of ~~Repressors~~ a biosynthetic pathway

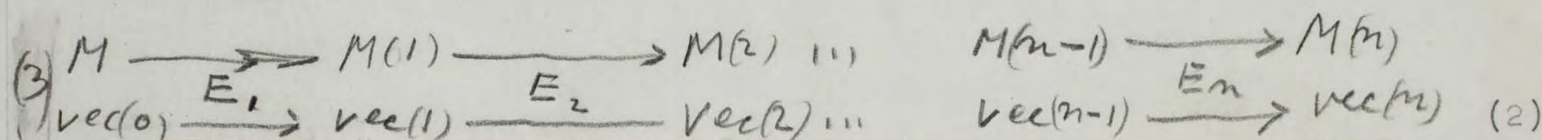
Enzymes are repressed ... [illegible]

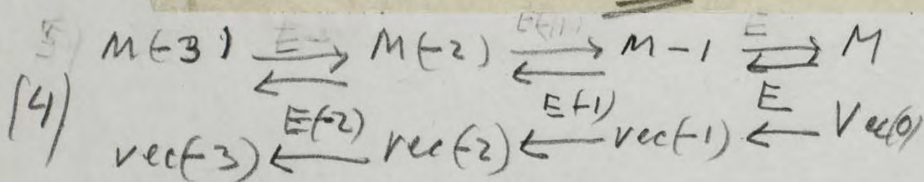
~~One of the basic concepts with which we shall operate is the concept of repressors, {M(n)-R,} which are composed of a specific metabolite, M(n), coupled to a radical, R, which we suspect to be a nucleotide or more likely a polynucleotide, composed of a few, perhaps three, nucleotides. The repressor, {M-R} contains the fundamental metabolite, M, which serves as a building block of~~

The Schematic biosynthetic Pathway

We shall adopt here a schematic biosynthetic pathway for simplifying communication with the reader. For the sake of simplicity, we disregard here and throughout the paper the existence of branched biosynthetic pathways with no other justification than to simplify communication. Accordingly, we shall represent by unbranched sequence the pathway which leads from an early metabolite, M(-n) to a fundamental metabolite, M, such as for instance an amino acid. The metabolic pathway leads beyond the metabolite, M. The metabolite, M, will be step by step modified through a series of enzymes, E(1) to E(n), etc., and will move in this manner towards its ultimate degradation. This is schematically represented by the following scheme.







The metabolic pathway (1) shows how a precursor, M(-n), of the metabolite, M, goes through a number of biochemical steps mediated by the enzymes E(-n), and ~~is~~ finally ~~converted~~ from a precursor, M(-1) ~~of the metabolite, M, by the enzyme, E, into the metabolite, M.~~

The biosynthetic step (2) shows how a coupling enzyme (or system of coupling enzymes), E_o, couples the metabolite, M, to a radical, R, and thereby converts it into the repressor, {M-R}. This repressor, also called vector, is designated by vec(0). The biosynthetic pathway (3) indicates how the metabolite, M, and the vector, vec(o) are transformed further, step by step by a sequence of enzymes, ~~E(1)~~x E_1 to E_n, etc., and thus move towards the ultimate degradation of the metabolite, M, and its vector vec(o).

~~We have assumed~~ here that the <u>same enzyme</u>, E_n, fulfills a double function; i.e. it transforms M(n-1) into M(n), and it transforms vec(n-1) into vec (n).

The symbol vec(n) strand for the compound, {M(n)-R}; ~~i.e. along the~~ metabolic pathway (3) the vector, R, remains unchanged whereas ~~towards~~ the moiety derived from the metabolite M undergoes a transformation. ~~By representing the metabolic pathway as we have above, we have implied that there is an~~ important difference between the ~~metabolites~~, M(-n) and the metabolite~~x~~, M(n). ~~which is a precursor of the~~ metabolite, M, can be converted into the metabolite, M, and ~~thereby also into the repressor, and also leads to the formation of~~ the compounds, {M-R} = vec(o)~~. which is a repressor for the formation of both the enzyme, E, that forms the metabolite, M, and the enzyme, E_o, that couples the metabolite, M, to a radical, R.~~ In contrast to this, a metabolite, M(n), ~~which lies~~ along the pathway of the degradation of the metabolite, M, ~~can~~ - ~~according to the scheme presented above, not be~~ converted into ~~a repressor.~~ As shall be seen later, ~~it is~~

this major difference between ~~percursors of the~~ prefundamental metabolite, M, (x) and most degraded or modified forms of the metabolite, M, ~~which permits us to understand~~ may explain why so many degradation products of ~~the~~ fundamental metabolite, M, ~~are able to~~ induce the formation of the enzymes which act upon them, whereas as a general rule precursors of the fundamental metabolite, M, ~~are unable to do so.~~ ~~do not~~ do not function as inducers.

The biosynthetic path (5) as represented here indicates that if the ~~steps~~ enzymatic steps ~~leading~~ leading say ~~from M(-3)~~ to the fundamental metabolite from the precursor its precursor say M(-3) is reversible then the ~~same~~ Enzymes involved may also ~~lead~~ ~~to the formation of the compounds~~ act on the corresponding repressors and may produce $\{M(-3)-R\}_3$, $\{M(-2)-R\}_2 = rec(-2)$, $\{M-1 - R = rec(-1)\}$ ~~Phage~~ with the ~~rec~~ $\{M-R\} = rec(0)$ $rec(-3)$ as starting material.

The first clear-cut case of a repression of enzyme synthesis was reported by Vogel. In the case of the biosynthesis of arginine = M, we have as a precursor of arginine, citrolin = M(-1), ornithin = M(-2), and acetyl ornithin = M(-3). Vogel has shown that the synthesis of the E(-3) is suppressed by the cells in a certain strain of Escherichia coli when this strain is grown in the presence of arginine. In the case of phenomena of this type we must, according to our theory, consider the reversible equilibrium, not only between the repressor and the template-enzyme-complex (see equations __________), but also the reversible equilibrium between the template-enzyme-complex of themetabolite, M, and its chemical analogues, M*, to which also belong the precursors of M. We indicate the ~~existence~~ existence of the equilibrium by writing (5)

E(2), etc. do not synthesize any essential building blocks. They might degrade metabolites or otherwise synthesize derivatives of metabolites. The metabolite M(n) may bear very little resemblance to M but it can be assumed to be a chemical analogue of M(n-1) and M(n+1). The same holds for the corresponding repressors which consist of the metabolite coupled to the carrier R. One may now ask why did all these enzymes, E(1) to E(2) come into existence during evolution. One way to derive the existence of such enzymes which do not synthesize any essential metabolite but merely chemical analogues of metabolites is as follows:

INSERT

Bacteria today having gone through a long evolution - so we assume - contain all these enzymes E(1) to E(n). Each enzyme E(n) produces a suppressor acting on its own enzyme template complex and acting on the enzyme template complex of the preceding enzyme E(n-1). Let us now assume that during evolution a mutation in the template T which synthesizes the enzyme E which produces the essential metabolite M. This mutation consisted in decreasing the equilibrium constant K(m) (i.e. which corresponds to an increase in the binding energy), and the repressor, rep, to the enzyme template complex. This is represented symbolically as follows:

(9)

One thus obtains a bug which can make only little enzyme E, and is therefore a slow grower. This may well have been compensated during evolution by a mutation that brought forth enzyme E(1) and the resulting repressor, rep(1), will then repress the production of the coupling enzyme E(0), and therefore reduce the output of the repressor, rep(0). This will then compensate for

decrease in the binding constant of rep(0) to the enzyme template complex. If now during evolution and subsequently the template T, that produces enzyme E, mutates once more and this time reverts back to its old form, this could have been compensated for during evolution by the appearance of enzyme E(2) which produces rep(2), and thereby represses production of enzyme E(1) which leads to the reduction in the output of rep(1), which in turn leads to an increase in output of rep(0), with the result that the back mutation is compensated for, at least as far as the production of enzyme (E) by the bacterium is concerned. Through such considerations one may make it plausible that the enzyme level in the bacterium is regulated in the manner described above and that for essential historical reasons today many genes have a voice in determining the level of a given enzyme E. These theoretical considerations are borne out by observations on suppressor mutations that occur with a functional gene which is specific for the production of the enzyme tryptophane synthesase. In a paper that appeared

Yanowsky, on the basis of his own work and the work of Bonner and Suskind, has reported on a number of mutatios occurring within this locus in Newospera. For a number of these mutations which led to loss of tryptophane synthesase activity and which, therefore, made tryptophane an obligatory growth factor, the tryptophane requirement was eliminated by subsequent mutation that occurred in another gene. This general phenomenon is called gene cooperation, and so far has defied an acceptable explanatiom. What made finding an explanation particularly difficult was the observation reported by Yanowsky that the suppressed mutations are highly specific. One suppressor gene might restore tryptophane independence to one or two of the mutants but

not to the others. This is represented in figure

(10)

Yanowsky furthher reports in this paper: " "

that all of his tryptophane-requiring mutants, for which there can be found a suppressor gene which can restore their ability to synthesize tryptophane, have one thing in common; that all produce a protein which is an immunological analogue of the enzyme though it possesses no enzymatic activity. We designate the immonological analogue of an enzyme andx a protein that may or may not be antigenic, but which reacts with the antibody evoked in rabbits by injection of the enzyme. Mel Cohn and Jacques Monod had previously reported that wild type coli, which produces a very low amount of β-galactosidase, contains an immunological analogue of β-galactosidase. The amount of this analogue is slightly decreased if the cells are highly induced and produce the enzyme β-galactosidase at a high rate. These results find their natural explanation on the basis of the notions here presented. In the bacterium in which the production of the enzyme is repressed, the enzyme template complex

the template is free from the enzyme only during an exceedingly small fraction of the time. If the enzyme template complex is protected by the inducer, the template might be free 10-20% of the time, but during the remainder of the time the enzyme is attached to the template. All we have now to assume is that there is a finite probability that during enzyme synthesis - during the time when the template is covered with the enzyme - a large fragment ~~fraction~~ of the polypeptide chain which is lined up along the whole lnngth of the template detaches itself to form a protein that does not contain the head of the enzyme which is combined by the repressor but may contain any

other parts of the enzyme. This fragment will then form a protein which is an immunological analogue of the enzyme. When enzyme production is highly repressed such debris can be produced almost 100% of the time, whereas if the enzyme is highly induced, it will be produced only 80% of the time.

We now turn our attention to a rather remarkable fact that many chemicals of small molecular weight, which do not have any close resemblance to the essential metabolites of the bacterium, can be metabolized by the bacterium and are metabolized by the enzymes which are induced in their presence. This is understandable if we assume that the chemical compound M* is a chemical analogue of some compound M(n) that is produced by the bacterium. Such a compound M* will then be metabolized by the enzyme E(n) as shown in Figure (8) just as the metabolite M(n) is metabolized by enzyme E(n). In addition, however, the compound M* being a chemical analogue of the compound M(n) will inhibit enzyme E(n), and thereby reduce the production of the repressor M(n)-R. As a result of this, the later production of the enzyme E(n) will be raised. Moreover by forming the complex

the compound M* will also diminish the repression of the synthesis of E(n). These facts are symbolically represented in Table (8).

We now turn our attention to the enzyme β-galactosidase, which is one of the most closely studied of the inducible enzymes. In the absence of an inducer, the level of this enzyme in the wild type strain of coli, which is used for most of these studies, is very low. In the presence of an inducer, high levels of enzyme will appear, and moreover mutants can arise from this strain which have an enzyme content that is several thousand times as high as the enzyme level of the wild type grown in the absence of inducer.

Because this enzyme acts on galactose derivatives and because it can be induced by certain artificially made β-galactosides; for instance, thio-methyl galactoside, we may assume that the repressor in this enzyme is a galactose derivative coupled with a carrier, R.

In the wild type and in the absence of an inducer we shall have the enzyme template complex, therefore, in two forms. In the presence of the inducer, gar*, the enzyme template complex will be present in three forms. By making the inducer concentration high, we can shift the equilibrium in such a manner that the concentration of enzyme template complex which is covered by R can be made low and the rate of production of the enzyme will then be high.

A mutation from the so-called inducible strain - wild type - to the so-called constitutive strain consists, according to our notion, in a change of the binding constant K_{rep}, so that this equilibrium constant is greatly increased in the constitutive mutant. This is symbolically indicated below

(12)

Since with increasing concentrations of TMG the rate of enzyme production increases faster than linearly TMG - according to the principles elaborated above - not only by protecting the enzyme template complex of this enzyme but also by either inhibiting an enzyme which produces the galactose derivative which -- coupled with the carrier acts as the repressor -- or else by enhancing the production of an enzyme which converts the gar derivative (or else the precursor of the derivative) into a harmless substance. Thiophenyl galactose which inhibits the formation of the enzyme β-galactosidase must act, according to our notions, by inhibiting an enzyme that converts, repressing galactose derivative or its precursor into something innocuous.

We now turn our attention to the problem of antibody production. I find that I am unable to understand this phenomenon as far as I can see in all its major aspects by assuming that cells in the lymphatic system of, say, rabbits are similar to bacteria, as well as inasmuch as they produce a great variety of enzymes $E(n)$, each of which is capable of transforming a metabolite which is capabiexof coupled to a carrier into another carrier-coupled metabolite so that we may write

because the number of genes in the mammalian cells might be much larger than in the bacterial cells, we are perhaps permitted to say that a variety of the compounds H and R could be somewhere between 1000 and 10,000. These compounds H and R are metabolized in the body with a short half-life of, say, perhaps a day, and the concentration, rn, of base equation

(13)

Enzymes $E(n)$ are made by some specific templates T, and the antibody-forming system contains the most important part at least of the template T which forms the enzyme. There is, however, this difference. Whereas templates T form a complete functional enzyme, the enzyme formed by the template contained in the antibody-forming side makes an aborted enzyme -- the antibody. Theantibody contains that part of the enzyme which is capable of combining with the compound $H(n)$, but it lacks that part which would enable it to convert $H(n-1)$ - R to $H(n)$-R. The repressors $H(n)$-R can diffuse everywhere and can both antibody template complexes as well as the enzyme template complexes.

the time, $\tau(AA)$, to assemble all the amino acids into a polypeptide and fold the polypeptide into an enzyme molecule. Let us further assume that after the enzyme molecules is formed it hangs on to the paragene and the paragene cannot synthetize additional enzyme molecules until the enzyme molecule already formed leaves. Let us now assume that the enzyme molecule can evaporate only while it is combined with the metabolite, M – or its precursor, M^-, or some suitable chemical analogues, M^*.

Within the framework of this model, we shall now consider either of two assumptions:

We may either assume

(a) that the molecule M that combines with the enzyme which sits on the paragene reevaporates at the rate $\frac{1}{\tau(M)}$, which is large compared to the rate $\frac{1}{\tau(E\text{-}M)}$, at which the enzyme-metabolite complex evaporates from the paragene. In this case we have $\tau(M) \ll \tau(E\text{-}M)$, and we obtain for the number of enzyme molecules per cell present in the steady state in a growing bacterial culture equation (1a)

$$(1a) \qquad N = \frac{\tau(\text{gen})}{\tau(E\text{-}M)\,\frac{1+\frac{[M]}{K_M}}{[M]/K_M} + \tau(AA)}$$

or we may assume (b)

(b)

(b)

~~or we may assume (b),~~ $\tau(M) >> \tau(E-M)$

and obtain equation (1b)

(1-b) $$N = \frac{\tau_{gen}}{\frac{1}{A_M \cdot [M]} + \tau(AA)}$$

In these equations the K_M designates the Michaelis constant for the combination of the metabolite, M, with the enzyme, E, and [M] designates the concentration of the metabolite, M. A_M is a [illegible] kinetic constant $A_M \cong 10^{11}$ if the concentrations are expressed in mol/cc

~~Both the equations (1-a) and (1-b) can be written in the form~~

~~(1-c) $$N = \frac{\tau_{gen}}{\varphi([M]) + \tau(AA)}$$~~

~~where $\varphi([M])$ is some function of [M]~~

~~We shall now consider N1 the quantity of an enzyme that has a high relative abundance in the cell and N2 the quantity of another enzyme that has a low relative abundance in the cell, and consider how in the steady state of a growing bacterial culture their ratio will change -- according to equation (1-c) -- if we first have a high growth rate so that τ(AA) is at its minimal value, and then slow the rate of growth so as to lengthen τ(AA) to twice its minimal value.~~

For paper

$$\tau = \frac{1}{A\rho}\,\frac{m}{1+\frac{\rho}{K}} + \frac{1}{\frac{A\rho K}{\rho}} \ln m \qquad \frac{\rho}{K} = x$$

$$\frac{1}{A\rho}\,\frac{m}{1+x} + \frac{x}{A\rho}\ln m \quad II \qquad \frac{1}{5}$$

77

$$\frac{1000}{12} = 84$$

$$-\frac{m}{(1+x)^2} + \ln m = 0$$

$$\frac{m}{\ln m} = (1+x)^2 \qquad m = 1000$$

$$x = \sqrt{\frac{m}{\ln m}} - 1 = 12 - 1 = 11$$

$$II \quad \frac{1}{AK}\ln m = \frac{1}{5}$$

assumptions of narrower $\frac{1}{35}$ sec

$$\frac{1}{A}\,35 = K = 12\;10^{-10} = 1.2\;10^{-9}\ \text{mol/cc}$$

$$\rho = \frac{1}{A}\,385$$

$$K = 1.2\;10^{-6}\ \text{mol/liter}$$

$$\rho = \frac{385}{3}\,10^{-10} = 1.3\;10^{-8}\ \text{mol/cc}$$

$$1.3\;10^{-5}\ \text{mol/lit}$$

$$I$$

$$\frac{1}{A\rho}\,\frac{m}{1+\frac{\rho}{K}} = \frac{1}{5}$$

$$\frac{1}{A\rho}\,\frac{5000}{12} = \rho = \frac{415}{A} = 1.4\;10^{-8}\ \text{mol/cc}$$

$$1.4\;10^{-5}\ \text{mol/lit}$$

August 30, 1957

APPENDIX

We make the following assumptions: $\tau(\text{ready})$

(1) For the fraction of time during which an enzyme sits on the paragene, whether covered or not covered by the repressor, we may write

(2) $$\tau(\text{ready}) = \frac{\tau\,\text{gen} - N\tau(AA)}{\tau(\text{free } E)}$$

And for the fraction of time during which an enzyme sits on the paragene and is not covered by a repressor, we may write $\tau(\text{cov } E)$

$$\tau(\text{free } E) = \frac{\tau\,\text{gen} - N\tau(AA)}{B}$$

(3)

The number of enzyme molecules produced per generation, N, is given by

$$N = \frac{\tau(\text{free } E)}{\tau(E)}$$

(4) $$\text{or } \tau(\text{free } E) = \tau(E)\,N$$

The time during which an enzyme sits on the paragene and is covered by a repressor, $\tau(\text{cov})$, is given by

$$\tau(\text{free}_E) \times A(R)[R](1-\pi) = \frac{\tau(\text{cov } E)}{\tau(R)}$$

(5)

where (6) $$(1-\pi) = \frac{1}{1 + \frac{[M]}{K[M]} + \frac{M'}{K[M']} + \frac{M^*}{K[M^*]}}$$

~~(6)~~

Taking into consideration the kinetic expression for the Michaelis constant, K(R)

(7) $$A\,K(R) = \frac{1}{\tau(R)}$$

we may write or $A R = A K \frac{R}{K} = \frac{[R]}{K(R)} \frac{1}{\tau(R)}$

We obtain from ~~(3), (4), and~~ (5)

(9) $$\tau(\mathrm{cov}) = \frac{[R]}{K(R)}(1-\pi)\cdot\tau(\mathrm{free})$$

~~(8)~~ and from this and (4)

(10) $$\tau(\mathrm{cov}) = \frac{R}{K[R]}(1-\pi)\,N\tau(E)$$

and from 3, 4, and 10 we obtain

(11) $$N\tau(E) = \tau(\mathrm{gen}) - N\,\tau(AA) - N\frac{R}{K(R)}(1-\pi)\tau(E)$$

$$N = \frac{\tau\,\mathrm{gen}}{\tau(E)\left(1+\frac{[R]}{K(R)}(1-\pi)\right)+\tau(AA)}$$

Time during which one enzyme site on polysome unit is covered by repressor ~~from (2)~~ ~~and from (2)~~ must be: $\tau(E)\frac{[R]}{K(R)}(1-\pi)$ ~~[illegible]~~ $\equiv \tau(\mathrm{cov})$ check: O.K.

~~14 $\tau(\mathrm{cov}) = \tau\,\mathrm{gen} - \tau(\mathrm{free}) - N\tau(AA)$~~ contd in new paper

Appendix

(1)

(2) fraction of time $= \frac{\tau_{gen} - S\,\tau(AA)}{\tau_{gen}}$

(3) fraction of time not covered =

$$F = \frac{\tau_{gen} - N\tau(AA) - \text{time covered}}{\tau_{gen}}$$

4) $N = \frac{F}{\tau(E)}\,\tau_{gen}$ ~~$= (\tau_{gen} - N\tau(AA)) - \text{time covered}$~~

S

(5) $g = F\,AR\,(1-\pi)$

where

6 $$(1-\pi) = \frac{1}{1 + \frac{[M]}{K(M)} + \frac{M^*}{K(M^*)}}$$

~~(7) $\frac{F}{\tau(E)}\,\tau_{gen}$~~

(7) ~~$N = \frac{\tau_{gen}}{\tau(E)\,[1 +}$~~

~~Following [illegible] [illegible] the [illegible] expression~~

(7) $$AR = \frac{1}{\tau(R)} \frac{R}{k}$$

(8) $$N = \frac{\tau\, gen}{\tau(E)\left(1 + \frac{[R]}{K(R)}(1-\pi)\right) + \tau(AA)}$$

$$\frac{N}{\tau(gen)} \simeq \frac{1}{\tau(E) + \tau(AA) + \frac{[R]}{K(R)}(1-\pi)}$$

~~for large R~~ ~~$\simeq \frac{1}{\tau(E) + \tau(AA)} \cdot \frac{1}{1 + \frac{R}{K(R)} \frac{(1-\pi)}{\tau(E) + \tau(AA)}}$~~

~~$\frac{1}{\tau(E) + \tau(AA)}$~~ . 1 −

for large $\frac{R}{K(R)}$

$$\frac{N}{\tau(gen)} \cong \frac{1}{\frac{R}{K(R)}} \quad \frac{}{\frac{\tau(E) + \tau(AA)}{\frac{R}{K(R)}} + 1} \sim \frac{}{}$$

$$\sim \left[1 - \frac{\tau(E) + \tau(AA)}{\tau(E)\frac{R}{KR}}\right] \times \frac{1}{\tau(E)\frac{[R]}{KR}}$$

1-Kinise cond ⟹ H

ΔA

$$\Delta\tau(E) = \frac{\tau_{gen}\left(1+\frac{R}{k}(1-\pi)\right) - \left[\tau_{gen} - S\tau(AA^*)\right]\frac{R}{k}(1-\pi)}{1+\frac{R}{k}(1-\pi)}$$

$$S\tau(E)\left(1+\frac{R}{k}(1-\pi)\right) = \tau_{gen}\left(1+\frac{R}{k}(1-\pi)\right) - \tau_{gen}\frac{R}{k}(1-\pi) + S\tau(AA)\frac{R}{k}(1-\pi)$$

$$S\left[\tau(E) + \frac{R}{k}(1-\pi)\left[\tau(E) - \tau(AA^*)\right]\right] \quad \tau_{gen}$$

$$\frac{\tau_{gen} + \tau_{gen}\frac{R}{k}(1-\pi) - \tau_{gen}\frac{R}{k}(1-\pi) + S\tau(AA)\frac{R}{k}(1-\pi)}{1+\frac{R}{k}(1-\pi)}$$

$$S\left(\tau(E)\left(1+\frac{R}{k}(1-\pi)\right) - \tau(AA)\frac{R}{k}(1-\pi)\right) = \tau_{gen}$$

$$S\tau(E) = \tau_{gen} - S\tau(AA) - \text{some cond.}$$

$$S\tau(E) = \tau_{gen} - S\tau(AA) - \frac{\tau_{gen}\frac{R}{k}(1-\pi) - S\tau(AA)\frac{R}{k}(1-\pi)}{1+\frac{R}{k}(1-\pi)}$$

$R^* = R(1-\pi)$

$$S\tau(E)\left(1+\frac{R^*}{k}\right) = \tau_{gen} + \tau_{gen}\frac{R^*}{k} - S\tau(AA)\left(1+\frac{R^*}{k}\right) - \tau_{gen}\frac{R}{k} + S\tau(AA)\frac{R^*}{k}$$

$$S\left(\tau(E)\left(1+\frac{R^*}{k}\right) + S\tau(AA)\right) = \tau_{gen}$$

$q = \frac{1 - s\tau(AA)}{}$

M

of in most cases covered

number of frequency at is $\frac{q}{\tau(R)}$

$\frac{q}{\tau(R)} \cdot \frac{\frac{1}{\tau(E)} + A(M)\, M}{A(R)\, R} = S$

$-(\alpha_1 + \alpha_2 + \alpha_3)t$

e

$$\frac{\frac{1}{\tau(E)} + \frac{1}{\tau(M)} A(M)[M]}{\frac{1}{\tau(E)} + \frac{1}{\tau(R)} + \frac{1}{\tau(M)}} = X$$

$$\frac{p - sp\tau(AA)}{\tau(R)} \cdot \frac{\frac{1}{\tau(E)} + A(M)\, M}{A(R)\, R} = S$$

$p - sp\tau(AA)\left(\frac{1}{\tau(E)} + A(M)\, M\right) = S\tau R \cdot A(R) R$

$$\left(\frac{p - sp\tau(AA)}{\tau(R)}\right) X = s$$

~~β p(of map) $\tau(E)$~~ $e^{-\alpha t}$

if dt inappropriate it takes time $\frac{1}{\alpha}$

~~$S\left(\frac{1}{\alpha} + \tau(AA)\right)$~~ $= T_{gen}$ ~~$= S\,\tau(AA)$~~

~~$\frac{1}{\alpha} = \frac{1}{\tau(E)} + A(R)\,R\,(1-\pi)$~~

~~$\frac{1}{\alpha} = \frac{\tau(E)}{1 + A(R)\,R\,(1-\pi)}$~~

number caught

~~$\tau(R)$ [illegible] $[T_{gen} - S\tau(AA)]\,A(R)\,R\,(1-\pi)$~~

time

~~number caught.~~

$\frac{R}{K}(1-\pi)$

~~time covered~~

$\left(T_{gen} - S\tau(AA) - \text{time covered with } R\right) A(R)\,R\,(1-\pi)\,\tau(R) =$

$\left[T_{gen} - S\tau(AA)\right]\frac{R}{K}(1-\pi) = \text{time covd}\left(1 + \frac{R}{K}(1-\pi)\right)$ = time covered

$\left[T_{gen} - S\times\tau(AA)\right]\frac{\frac{R}{K}(1-\pi)}{1 + \frac{R}{K}(1-\pi)} = \text{time covd}$

[illegible] $- S\tau(AA)$

$\frac{1}{\tau(E)}\left(\text{[illegible] time covd}\right) = S$

$$\cancel{N} = \frac{\overline{U}_{gen}}{\tau(E)\left[1 + \frac{[R]}{K(R)}(1-\pi)\right] + \tau(AA)}$$

$$1 - \pi = \frac{1}{1 + \frac{[M]}{K(M)} + \frac{[M^*]}{K(M^*)}}$$

before S was written for N
and R^* for $R(1-\pi)$

Jachson Ich antwortete ~~prompt~~
Ungarisch natürlich ..... ? Natürlich
spreche ich ungarisch antwortete mein
engl. Kollege ich ~~aber~~ nicht ~~ist~~ verstehe ~~nicht~~ (ohne mit der Wimper
zu zucken.

$S*\left(1 - \text{"}\text{list}_4\right)$ + [illegible] M^*

prob. $\left[A(R)\, R\, (1-\pi)\ \tau(R) + \text{probev ov}_x\ \tau(E) \right] S$

$\left[AR(1-\pi) + \frac{1}{\tau(E)} + \tau(AA) \right] S = \tau_{gen}$

1.) probutab being [illegible] $= \dfrac{A(R)R(1-\pi)}{A(R)R(1-\pi) + \frac{1}{\tau(E)}}$ $\| \tau(R)$

2.) probabl of map $\dfrac{1/\tau(E)}{A(R)R(1-\pi) + \frac{1}{\tau E}}$ $\| \tau(E)$

3.) $\tau(AA)$

(1) $\dfrac{\frac{1}{\tau R} A(R) K \frac{R}{K} (1-\pi)\, \tau(R)}{\frac{1}{\tau(R)} A(R)(K) \frac{R}{k}(1-\pi) + \frac{1}{\tau(E)}} = \dfrac{\frac{R}{k}(1-\pi)\, \tau(R)\, \tau(E)}{\tau(E) \frac{R}{K(R)}(1-\pi) + \tau(R)}$

$\tau_{p\,gen} = S \left(\dfrac{A(R)\, R(1-\pi)\, \tau(R)}{A(R) R(1-\pi) + \frac{1}{\tau(E)}} + \dfrac{1}{\tau(A(R) R(1-\pi) + \frac{1}{\tau_E})} \right)$

$= \left[\frac{R}{K(R)}(1-\pi)\frac{1}{\tau(R)} + \frac{1}{\tau(E)} \right]$

$S = \tau_{gen}\ \dfrac{\ }{\frac{R}{K(R)}(1-\pi) + 1 + \frac{\tau(AA)}{\tau(R)}} \quad \left(\frac{R}{K(R)}\right)(1-\pi) + \frac{\tau(AA)}{\tau(E)}$

$S = \tau_{gen}\ \dfrac{\frac{R}{K(R)}(1-\pi)\frac{\tau(E)}{\tau(R)} + 1}{\left[\frac{R}{K(R)}(1-\pi) + 1 + \frac{\tau(AA)}{\tau(R)} \right] \tau(E) + \tau(AA)}$

August 30, 1957

APPENDIX

We make the following assumptions:

(1) ~~(1)~~ For the fraction of time during which an enzyme sits on the paragene, whether covered or not covered by the repressor, we may write

(2)

And for the fraction of time during which an enzyme sits on the paragene and is not covered by a repressor, we may write

(3)

The number of enzyme molecules produced per generation, N, is given by

(4)

The time during which an enzyme sits on the paragene and is covered by a repressor, G, is given by

(5)

where

(6)

Taking into consideration the kinetic expression for the Michaelis constant, K(R)

(7)

We obtain from (3), (4), and (5)

(8)

Memo to page 11 of draft of August 28, 1957

Appendix to Enzyme paper.

~~N = τ(E)~~ XY –

Equations ~~(19) and (20)~~ can be written ~~in the case of strong repression where the term, τ(AA), can be included also as follows~~ neglecting and where τ(E) << τ(AA) as follows

$$(1) \quad N \cong \frac{\tau\, gen}{\tau(E)\, \frac{R}{K(R)}\, \frac{1}{1+\frac{M}{K(M)}+\frac{M^*}{K(M^*)}}} + \tau(AA)$$

~~which we may write also in the form~~

$$A(R)K(R) = \frac{1}{\tau(R)} \quad \text{or} \quad K(R) = \frac{1}{\tau(R)AR}$$

According to the kinetic theory of the equilibrium, we may write ~~for K(R), τ(R)/A~~ ~~A(R) K(R) = τ(R) or K(R) = τ(R)/A(R)~~, where A represents the number of successful hits of the molecule, R, against the uncombined enzyme template complex that leads to the formation of the enzyme template repressor complex, and τ(R) the average time in tubes for the repressor R to evaporate from the [illegible] – Enzyme complex so that we obtain

$$(A) \quad N \approx \frac{1/\tau(E)}{\tau(R)AR\, \frac{1}{1+\frac{M}{KM}+\frac{M^*}{K(M^*)}}} + \tau AA \quad ** \frac{\tau\, gen}{\tau(R)}$$

first [illegible]

number of [illegible]

$$(XY) \quad N = \frac{\tau\, gen}{\tau(E)\left(1+\frac{R/K(R)}{1+M/K(M)+\frac{M^*}{KM}}\right)} + \tau AA$$

~~neglecting τ(AA) and [illegible] τ(E) << τ(AA)~~

If we deal with the strongly repressed enzyme, we can neglect in this formula the term $\tau(AA)$, and we obtain the following:

$$N = \frac{\tau(gen)}{\tau(R)} \times \frac{1/\tau(E)}{AR \dfrac{1}{1+\frac{M}{K(M)}+\frac{M^*}{K(M^*)}}}$$

We can see the physical meaning of this formula as follows: If the enzyme is strongly repressed, the paragene spends most of its time on the paragene-enzyme repressor complex. The first factor in this formula, $\tau gen/\tau(R)$, give the number of times that a repressor will evaporate from this complex leaving a paragene-enzyme complex uncovered by the repressor. The second factor of this formula gives the probability that when the repressor evaporates from the complex leaving the enzyme on the paragene, the enzyme will evaporate before the paragene-enzyme complex combines with the repressor. This may be seen as follows:

The expression $\frac{1}{1+\frac{M}{KM}+\frac{M^*}{K(M^*)}}$ describes the probability that, the paragene-enzyme complex which is not combined either with the metabolite, M, or the inducer, M*. The denominator as a whole represents the rate at which the paragene-enzyme complex, which is free from the repressor, is complexed with the repressor. The nominator, on the other hand, $1/\tau(E)$, represents the rate at which the enzyme, when uncombined with the repressor, evaporates from the paragene-enzyme complex.

tain the same information as does the gene, and I shall refer to it, therefore, as the paragene.

There is a maximum rate at which a paragene can synthetize the enzyme when it is producing the enzyme at a full rate, say, in a strongly induced cell. I assume that when the cell is induced the number of paragenes synthetizing that enzyme do not increase, but what increases is rather the fraction of the time during which the paragene which makes the enzyme for which it is specific. How can this be accomplished?

Since the metabolite, M, and its precursor and their chemical analogues all have in common the ability to combine with the enzyme, it one is naturally tempted to try to explain enzyme induction by reasoning as follows: Let us assume that when the paragene makes enzyme at the full rate, it takes the time, τ (AA) , to assemble all the amino acids in a polypeptide and fold the polypeptide into the enzyme. Let us further assume that after the enzyme it forms, it hangs on to the paragene and the paragene cannot synthetize additional enzyme molecules until the enzyme molecule already formed leaves. Let us now assume that the enzyme molecule will leave only if it is complexed by the precursor, M -- the metabolite, M^-, or some suitable chemical analogues, M*, of these metabolites.

It is clear that the rate of enzyme production can then be increased by increasing the concentration of the inducers, M^-, or M*. This gives for the number of molecules of a specific enzyme present per cell, when the inducer, M^*, is present in a concentration, M^*, and when K designates the Michaelis constant for the combination of the inducer with the enzyme.

This formula is, however, in gross contradiction to what we may call the principle of growth-rate independence of enzyme composition. Under natural

conditions the bacteria rarely grow at their maximal rate. There is usually some growth factor which is present in low concentration and slows the growth of the bacteria. In a bacterium which grows at its optimal (maximum) rate, some of the enzymes will be present in high abundance and all others there will be just a few molecules per cell in the steady state of the growing culture. If the growth rate is slowed to half because, say, an amino acid which the bacterium requires as a growth factor is present in a low concentration, the equation 1 which we obtained demands that both enzymes will now be present in about the same concentration. This contradicts the law of growth-rate independence which we just postulated.

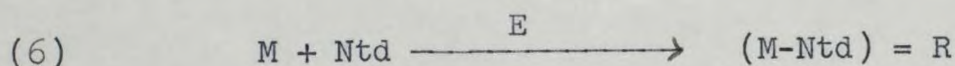
The enzyme, β-galactosidase, which splits lactose, has been extensively studied by Jacques Monod and his collaborators in Paris, and more recently also by Aaron Novick and Milton Weiner. In many of these experiments the bacteria were grown in the Chemostat where the rate of protein synthesis is controlled through the supply to the bacteria of one amino acid, say, for instance, tryptophan, which the bactertia require as a growth factor. When the enzyme is fully induced, there are about 10,000 molecules of this enzyme persent per bacterium, whereas on the average there are only 60 molecules of an enzyme present in the steady state of the growing culture. In different experiments the bacteria were grown in the Chemostat at different rates, and in the same experiments the rate of growth was slowed by a factor of 2 in the middle of the experiment. A fall in the enzyme content of a highly induced cell was never observed in such experiments when the growth rate was cut in half. The above postulated principle of growth-rate independence appears, therefore, to hold for this well studied enzyme.

Both because of this difficulty and also because it is difficult to visualize a mechanism whereby an inducer, such as the metabolite, M, the precursor, M^-, or their chemical analogues, M*, could entice the enzyme which hangs on to the paragene to leave the paragene. This first try must be abandoned.

As the next try, we may now assume that there are present in the cell metabolites, which we shall call repressor molecules, R, and which -- as we shall describe presently in greater detail -- can combine with the paragene-enzyme complex in a reversible manner. As long as the paragene-enzyme complex is thus covered by the repressor, the enzyme cannot evaporate.

What kind of molecule must the repressor molecule, R, be in order to be able to prevent the evaporation of the enzyme molecule which has been formed from the paragene? We assume that such a repressor molecule contains a moiety that can combine with the enzyme, such as a metabolite, M(O), and it must contain a moiety, Ntd, which can combine with a certain part of the paragene -- perhaps a group of nucleotides on the paragene. It is conceivable that the moiety, Ntd, might itself be composed of nucleotides; hence the designation, Ntd. For such a molecule, R, to function as a repressor, it is necessary that it "fit" the paragene-enzyme complex in the sense that the moiety, M, must be able to hold on to the enzyme at the same time as the moiety, Ntd, holds on to the paragene. This must impose specific conditions on the spatial configurations of the repressor, R.

Such a repressor molecule, R, may be formed in the cell from the metabolite, M, by the reaction



$$(6) \qquad M + Ntd \xrightarrow{E} (M\text{-}Ntd) = R$$

The enzyme, E*, may be called "coupling enzyme" and such a coupling enzyme, as well as the moiety, Ntd, might be specific for each metabolite, M, although a number of metabolites along the same biochemical pathway might be able to react with the same coupling enzyme and the same Ntd moiety.

We shall now define as a real inducer (in contradistixtinction to quasi-inducers -- to be defined later) a chemical compound, M*, that is a chemical analogue of a metabolite, M (which is produced by the enzyme, E, or its immediate precursor, M') and that can combine with the enzyme molecule that sits on the paragene but cannot be transformed by the cell into a repressor. Such a real inducer will always enhance the formation of the enzyme by reversibly combining with the enzyme moiety of the paragene-enzyme complex because during the time when the enzyme is occupied by the inducer, M*, the paragene-enzyme complex is protected from being covered by a repressor molecule. The enzyme which is thus combined with the inducer, M*, evaporates off the paragene at the same rate, $1/\tau(E)$, as the enzyme which is uncombined with anything.

If the enzyme which sits on the paragene is combined with the metabolite, M or M', it will also evaporate at the same rate, $1/\tau(E)$, and the metabolite, M or M', also protects the paragene-enzyme complex against being covered by the repressor. However, this effect of the metabolite, M or M', may or may not be counterbalanced by the fact that the cell can transform the metabolite, M or M', into a repressor, and the metabolite, M or M', may therefore raise the concentration of the repressor molecules, R, in the cell. Accordingly, the metabolite, M or M', may, depending on the circumstances, either enhance the formation of the enzyme or repress it, in contradistinction to the inducer, M*, which will always enhance enzyme production. "In cases where a metabolite will enhance the formation of an enzyme, we shall designate it as a "quasi-inducer."

There is another difference between M (or its precursor) and M*. Where M or M' as well as M* may enhance enzyme formation directly by combining with the enzyme, E, sitting on the paragene and thereby protecting the paragene enzyme complex from being covered by the repressor, the real inducer, M*,

can in addition enhance the formation of the enzyme <u>indirectly</u>. It can do this in two ways. M* by competing with the metabolite, M'(n) for the enzyme may enhance the formation of the enzyme, both by raising the concentration of the metabolite, M', and by decreasing the concentration of the metabolite, M, from which the cell may form the repressor, R.

The combination of the metabolite, M or M', and the inducer, M*, with the enzyme is reversible and so is the combination of the repressor, R, with the paragene-enzyme complex.

The model here adopted assumes that when the repressor evaporates from the paragene-enzyme complex it leaves the enzyme on the paragene.

Having thus described qualitatively a model for enzyme induction and repression, we shall now proceed to compute, N, the number of molecules of one specific enzyme per cell which may be maintained in a growing culture, depending on the concentrations of the compounds, M, M', M*, and R, which are maintained in the cell. These concentrations, M', we shall designate by $[M]$, $[M']$, $[M^*]$, and $[R]$. For the Michaelis constants of these compounds, we may write K(M), K(M'), K(M*), and K(R), respectively. Similarly we shall designate the average time which it takes for M and M* to evaporate off the enzyme and for R to evaporate off the paragene-enzyme complex, and for the enzyme to evaporate off the paragene with $\tau(M)$, $\tau(M')$, $\tau(M^*)$, $\tau(R)$, and $\tau(E)$, respectively.

In order to be able to give a concrete formula for N', the number of molecules of a given enzyme maintained per cell in the growing culture, it is necessary to make certain assumptions concerning the evaporation times, $\tau(M)$, $\tau(M')$, $\tau(M^*)$, $\tau(R)$ and $\tau(E)$. Our assumptions will be as follows:

(7) $$\tau(R) >> \tau(E)$$

(8) $$\tau(M),\ \tau(M'),\ \tau(M^*) << \tau(E)$$

For reasons that will be stated later, we shall also assume

$$\tau(E) << \tau(AA)$$

The times $\tau(M)$, $\tau(M^*)$ and $\tau(R)$ may be roughly estimated by writing

$$\frac{1}{\tau} = 10^{13}$$

where $\Delta H(M)$, $\Delta H(M^*)$ and $\Delta H(R)$ represent the binding energy of the compounds to the enzyme or enzyme-template complex, and by making a rough estimate of these binding energies we may surmise that we have $\tau(M') < \frac{1}{100}$ sec; $\tau(M^*) < \frac{1}{100}$ sec; $\tau(R) >> 1$ sec. For $\tau(AA)$, we obtain from a rather rough consideration $\tau(AA) \sim 1$ second.

We have no way of estimating directly $\tau(E)$ but since, for reasons to be stated later, we are forced to assume that $\tau(E) << \tau(AA)$, we may hazard as a rough guess that we might have perhaps $\tau(E) \sim \frac{1}{10}$ sec.

Assuming that inequalities 7, 8 and 9 hold, we obtain for N the number of molecules of a given enzyme that is maintained in the cell in the growing culture

(9) $$N = \frac{\tau_{gen}}{\tau(E)\ (1 + \frac{(R)}{K(R)}\ (p) + \tau(AA)}$$

where

(10) $$p = \frac{}{1 + \frac{M}{K(M)} + \frac{M'}{K(M')} + \frac{M^*}{K(M^*)}}$$

For high values of M*; i.e. if the cell is fully induced, we obtain from equation (   ) N_{max}

(11) $$N_{max} = \frac{\tau_{gen}}{\tau(E) + \tau(AA)}$$

As we have mentioned before, in a fully induced cell there are about 10,000 molecules of the enzyme; i.e. we may put N_{max} = 10,000. Accordingly, for a cell which is growing in minimal medium, supplemented with tryptophan, at a fast rate, so that we have $\tau(gen)_{min}$ = about 5,000, we obtain from (   ) $\tau(E) + \tau(AA)$ = 1/2 sec., and since we are assuming $\tau(E) < \tau(AA)$, we obtain $\tau(AA)_{min}$ = 1/2 sec.

If we want to see how the ratio of the amount of a highly induced enzyme and the amount of a slightly induced enzyme changes when raise the time, $\tau(AA)$, from its minimal value to double its value, we write - as we did before - the ratios

(12) $\dfrac{\text{Highly induced}}{\text{Slightly induced}} =$

If we assume that the concentration of the repressor, R, does not change when we double the time, $\tau(AA)$, then we find, as we did before

This is the same flagrant violation of the principle of growth-rate independence of enzymatic composition. There is an avenue of escape from this conclusion but there is serious doubt that nature has taken this particular avenue of escape. One might argue that perhaps the repressor molecule, R, is not further metabolized by the cell, and in that case, if it is produced at a rate which is independent of $\tau(AA)$ in the steady state in a growing culture, its concentration may be proportionate to $\tau(AA)$. $\tau(AA)$, τ_{gen}, and R would then all rise by the same factor if the generation time is changed, and the enzyme level maintained in the cell would indeed be independent of the generation time. In that case one should expect the following: When the culture is grown fast in the Chemostat and the generation time is then double at a given point in time, it will take a generation time until the concentration of the repressor, R, rises 63% towards its final value. During this time there would be a rapid fall in the enzyme level, even though

after a while the enzyme level will xx turn back at the high generation time to the same point at which it was at the low generation time. I believe that this effect would most probably have been induced in the course of the many experiments which were performed by Milton Weiner and Aaron Novick on the enzyme, β-galactosidase, in bacteria growing in the Chemostat. In order to be certain about this point, it will be necessary to repeat some of these experiments. Pending proof to the contrary, we may assume that the enzyme level will not change when the generation time is doubled even temporarily. As far as I can see all of the models which can reasonably be suggested, there is only one solution to the dilemma in which we find ourselves that leads to a model that is both reasonable and obeys the principle of the growth-rate independence composition, which models 1 and 2 violate. This model 3 is in every respect the same as model 2 except in one respect. According to model 3, when a repressor molecule, R, combines with the paragene-enzyme complex, it does not leave it again. At the time when the bacterium divides two new paragenes are formed, either the paragene-enzyme repressor complex is then dissociated and another paragene formed at the same time, or else the paragene which is complexed with the paragene repressor is destroyed and two paragenes are created _de novo_. In order for this to be possible, we must demand that the binding energy of the repressor molecule to the paragene-enzyme complex be large enough to permit the repressor molecule to remain complexed even when $\gamma(AA)$ is increased from its minimal value to _____fold, corresponding to a generation time of perhaps 12 hours. We shall further below examine this proposition m more closely. According to model 3, after a new paragene has been created, it will make for a period of time the enzyme at the full rate. Each time an enzyme is made there is for the period, $\gamma(E)$, a certain probability, p, that the repressor will combine with the paragene-enzyme complex, and in that case that paragene will never again make this enzyme. In this case one

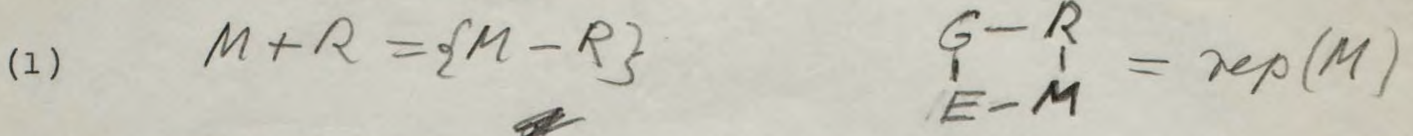
may see without going through any arithmetical computation that if p is ________ and ________ ----- ; for instance if **N**(0) is 10%, p is 1/10th or 1/100th, the number of enzyme molecules is given by

(13)

Ist draft

Theory of Repression

A metabolite produced by an enzyme may repress the formation of an enzyme if it undergoes the reaction



(1) $M + R = \{M - R\}$ $\begin{matrix} G - R \\ | \quad | \\ E - M \end{matrix} = rep(M)$

If the spatial configuration is such that in the molecule rep(M) the moiety M can combine with the enzyme at the same time when the moiety R can combine with the head of the paragene then $\{M-R\}$ is a repressor.

If the equilibrium constant K(rep) which controls the combination of rep with the paragene-enzyme complex is of the order of magnitude of 10^{-9} Mol/l then the time it takes for rep to evaporate from the paragene enzyme complex is about 10 seconds. (This compares correctly with the evaporation time of 10^{-1} second of the trinucleotides from the paragene at $K = 10^{-7}$ Mol/l.

During a generation time of $\tau_{gen} =$ 4,000 seconds on a strongly repressed paragene there will be about $400 = \tau_{gen}/\tau_{rep}$ acts of evaporation of rep. We shall further assume that the evaporation time $\tau(E)$ of the enzyme is of the order of magnitude of 1/10 second, and incidentally we shall also assume that, when growth takes place in minimal medium at a generation time of 70 minutes, the time it takes to synthetize the enzyme; i.e. $\tau(AA)$ is about 1 sec.

Let us now estimate the concentration of the repressor on the assumption that 4 enzyme molecules are produced per generation. There are 400 chances in a generation that an enzyme molecule might be produced, because the repressor sits each time for about 10 seconds on the paragene. On each such occasion if no repressor molecule hits the enzyme during a time period of τ_E an enzyme molecule is produced. According to the Poisson formula

Correction:

rate of enzyme formation

$$S = \frac{1}{\tau_{gen}} \frac{1}{\alpha} \frac{1}{\tau_{rep}} \times \frac{\tau(E)}{A_{rep} \rho_{rep}} =$$

or per generation

N = enzyme level $= S\tau_{gen} = \frac{\tau_{gen}}{\tau_{rep}} \frac{\tau(E)}{A_{rep}\rho_{rep}} = N$ $\frac{1}{4}$ $\frac{1}{400}$ $1/10$

or if we set:

$S\tau = 4$

$A_{rep}\rho_{rep} = \frac{10}{100}$

$A = 10^{10}$

$\rho = 10^{-9}$ mol/cc $= 10^{-6}/l$

~~$(\rho(M) = 10^{-5}$ mol/l$)$~~

~~$A_{rep}K_{rep} = \tau(E_{rep}) \frac{1}{\tau_{rep}}$~~

$\tau_{rep} = \frac{1}{AK}$

$K = 10^{-12}$ mol/cc

~~$10 \times K = 10^{-11} =$~~

$A = \frac{1}{K\tau_{rep}}$

$$N = \frac{\tau_{gen}}{\tau_{rep}} \frac{\tau_w(E)}{\frac{V}{\tau_{rep}} \frac{\rho_{rep}}{K_{rep}}}$$

thw probability that this will happen is given by

(2)

where rep

(3)

Accordingly the number of enzyme molecules formed per generation is given by

(4)

and if we demand that four molecules be formed in one generation, then we have

(5)

If $\tau(E)$ is equal to 1 second, we obtain

(6)

and if we assume for A

(7)

we obtain four________.

For s,) the rate of enzyme production we may write

(8) $$s = \frac{1-p}{\tau_{ev}(E)} \times q$$

$$\rho = \frac{\frac{\rho_{rep}}{K_{rep}}}{1 + \frac{y}{K(y)} + \frac{\rho_{rep}}{K_{rep}}} \quad \Big| \quad 1-\rho = \frac{1 + \frac{\rho(y)}{K(y)}}{1 + \frac{\rho(y)}{K(y)} + \frac{\rho_{rep}}{K_{rep}}}$$

3.

p is the probability that enzyme is combined with the repressor. q is probability that an enzyme molecule is sitting on the paragene and we may write for q

(9) $$q = 1 - s\tau(AA)$$

From (8) and (9), we obtain

(10) $$\frac{1}{s} = \frac{\tau_{ev}(E)}{1-p} + \tau(AA)$$

$$s = \frac{1}{\frac{\tau_{ev}}{1-p} + \tau(AA)}$$

$$\frac{1}{1-p} = 1 + \frac{R}{K(R)}$$

And for 1-p, we may write

$$N = s\tau_{gen} = \frac{1}{\frac{\tau_{ev}}{\tau_{gen}(1-p)} + \frac{\tau(AA)}{\tau_{gen}}}$$

(11) $$1 - p = \frac{1 + \frac{y}{K(y)}}{1 + \frac{y}{K(y)} + \frac{\rho_{rep}}{K_{rep}}}$$

If we have an internally made repressor which accumulates, its concentration will be inversely proportionate to the growth rate, α, provided it is produced by a strongly repressed enzyme which, as we shall see, is present in the concentration which is independent of the growth rate. That this is so is consistent with equation (10) from which we see the following: If we double $\tau(AA)$ and if the growth rate falls to half the rate of enzyme production s falls to half and 1/p falls to half provided

$\rho_{rep} = BN\tau_{gen}$

(12) $$y = 0 \quad \frac{\rho_{rep}}{K_{rep}} \gg 1; \text{ so that } \frac{1}{1-p} = \frac{\rho_{rep}}{K_{rep}}$$

Therefore a strongly repressed enzyme may be expected to have a concentration which is independent of the growth rate, and a non-repressed enzyme ($p = 0$) will also behave in the same way, provided that we have

(13) $$\tau(AA) \gg \tau_{ev}(E)$$

$$N = \frac{\tau_{gen}}{\tau_{ev} + \tau(AA)}$$

3f $\frac{g_{rep}}{K_{rep}} \Big/ \frac{y}{K(y)} >> 1$

15 C

$$N = \frac{\tau_{gen} \times \frac{y}{g_{rep}} \frac{K(rep)}{K(y)}}{\tau_{ev}}$$

$$N = \tau_{ev}\left(1 + \frac{g_{rep}}{K_{rep}} \Big/ \frac{y}{K(y)}\right) + \tau(AA)$$

15B

$$N = \frac{\tau_{gen}}{\tau_{ev} \times \frac{g_{rep}}{K_{rep}} \Big/ \frac{y}{K(y)} + \tau(AA)}$$

Induction

If we have a metabolite, I, present which combines with the enzyme, then we may write

$$(14) \quad 1-p = \frac{1 + y/K(y)}{1 + \frac{y}{K(y)} + \frac{\rho_{rep}}{K_{rep}}} \qquad \Bigg| \qquad \frac{1}{1-p} = \frac{1 + \frac{y}{K(y)} + \frac{\rho_{rep}}{K_{rep}}}{1 + \frac{y}{K(y)}}$$

or for the ~~rate of~~ enzyme level ~~production~~ we will then obtain

$$(15) \quad N = \tau_{gen} \frac{1}{\tau_{cw} \times \frac{1 + y/k + \rho/k}{1 + y/k} + \tau(AA)}$$

or if the inducer is produced internally by ~~a~~ strongly suppressed enzymes, its concentration will be inversely proportional to the growth rate, and if we have

$$(16) \quad \frac{y}{K(y)} \gg 1 \quad \text{and} \quad \frac{\rho_{rep}}{K_{rep}} \gg 1$$

then the first term in (denominator of) (15A) does not change with the growth rate, and if, further, this first ~~step~~ term is larger compared to the second, ($\tau(AA)$), then ~~when~~ we ~~slow the growth rate we~~ have

$$(17) \quad N = \frac{\tau_{gen}}{\tau_{cw} \left(1 + \tau(AA)\right) \frac{\rho_{rep}}{y} \times \frac{K(y)}{K_{rep}}}$$

(original: $N = \tau_{gen} \frac{1}{\tau_{cw}} \cdot \frac{y}{k} \Big/ \frac{\rho_{rep}}{K_{rep}}$)

which means that the enzyme level goes up ~~inversely proportional with α.~~ not proportional to τ_{gen}

¶ On the other hand if we have an outside inducer I = constant independent of α, then in equation (17) the denominator increases with ~~rho-rep~~ ρ_{rep} (i.e. with $\frac{\tau}{\tau_{gen}}$) and the enzyme level is independent of the growth rate.

If we have a metabolite, M, which is supplied in the nutrient, which can combine with the enzyme and which can be converted by the cell into a repressor, we must distinguish three different cases:

$$(15A) \quad N = \tau_{gen} \frac{1}{\tau_{cw}\left(1 + \frac{\rho_{rep}/K_{rep}}{1 + \frac{y}{K(y)}}\right) + \tau(AA)}$$

~~$N \cong \tau_{gen} \times \frac{\quad}{\tau_{cw}}$~~

a) If the repressor concentration [R] is proportional to the metabolite concentration, M the metabolite will neither enhance nor repress the formation of the enzyme, ~~prxxxd~~ provided that

(18) $$\frac{M}{K(M)} >> 1 \qquad \frac{R}{K(M)} >> 1$$

This may be seen from equation (~~15B~~ 15B)

6) If the repressor concentration rises more slowly than the metabolite concentration, the metabolite will act as an inducer ~~and~~

because of same kind of saturation of the conversion of the metabolite to the repressor then

7) If we have

ρ_{rep} proportional to M

(~~19~~) 20 and $$\frac{M}{K(M)} << 1$$

than the metabolite will act as a repressor. see

see 15A. —

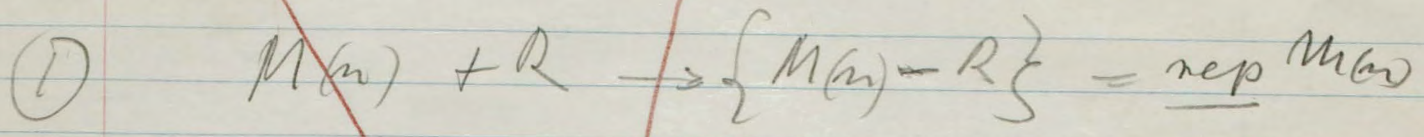
if $\frac{\rho_{rep}}{K_{rep}} \Big/ \frac{y}{k(p)} >> 1$ we may write

from 15B

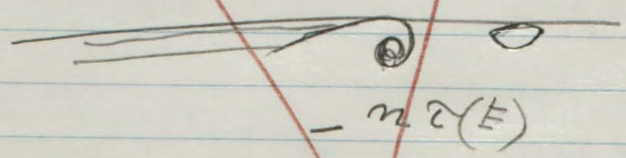
19 $$N \cong \frac{\frac{M}{K(M)} \tau_{gen}}{\tau_w \frac{\rho_{rep}}{K_{rep}} + \tau \text{[crossed out]}} \pm \text{[crossed out]}$$

[crossed out] $$= \frac{\tau_{gen} \; \frac{M}{\rho_{rep}} \frac{K_{rep}}{K(M)}}{\tau_w}$$

old



(1) $M(n) + R \rightarrow \{M(n) - R\}$ = rep $M(n)$



(2) $e^{-n\tau(E)}$

where

3 $n = A\rho$

4 $\delta \frac{1}{\alpha} = 400\, e^{-n\tau(E)}$

5.) $\delta \frac{1}{\alpha} = 400\, e^{-n\tau(E)} = 4$

$\tau(E) = 1$ sec

we obtain

6 $n = A\rho =$

7 $A = 10^{10}$

we obtain for $\rho = 10^{-9}$ mol/cc or 10^{-6} mol/cc

8 $\frac{1}{\tau} = S = \frac{(1 - p)}{\tau(E)} \times$ (probability that enzyme is sitting there) [q]

p is probability that enzyme is combined with repressor

9 $q = 1 - S\tau(AA)$

pump and of mechanism

$$N = \frac{\tau_{gen}}{\frac{\tau_{er}}{1-p} + \tau(AA)},$$

$$S = \frac{1}{\frac{\tau_{er}}{1-p} + \tau(AA)}$$

10) $$\frac{1}{S} = \frac{\tau(E)}{1-p} + \tau(AA)$$

11.) and $$1-p = \frac{1}{1+\frac{\rho_{rep}}{K_{rep}}} \approx \frac{K_{rep}}{\rho_{rep}}$$

provided

12.) ~~provided~~ $\left(\frac{\rho_{rep}}{K_{rep}} \gg 1 \text{ and } \frac{1}{1-p} = \frac{\rho_{rep}}{K_{rep}}\right)$

13.) $$\tau(E) < \tau(AA)$$

14.) $$1-p = \frac{1+y/K(y)}{1+\frac{y}{K(y)}+\frac{\rho_{rep}}{K(rep)}}$$

or

$$\frac{1}{1-p} = \frac{1+\frac{y}{K(y)}+\frac{\rho_{rep}}{K_{rep}}}{1+y/K(y)}$$

15) $$\frac{1}{S} = \tau(E)\,\frac{1+\frac{y}{K(y)}+\frac{\rho_{rep}}{K_{rep}}}{1+\frac{y}{K(y)}} + \tau(AA)$$

or

15a $$\frac{1}{S} = \tau(E)\left(1+\frac{\rho_{rep}/K_{rep}}{1+\frac{y}{K(y)}}\right) + \tau(AA)$$

and if we have:

16 $$\frac{y}{K(y)} \gg 1 \quad\text{and}\quad \frac{\rho_{rep}/K}{~~y/K(y)~~} \gg 1$$

17 $$\frac{S}{\alpha} \cong \frac{1}{\alpha}\,\frac{1}{\frac{\rho_{rep}/K_{rep}}{y/K(y)} + ~~\tau(AA)~~}$$

18) $\frac{\delta M}{K(M)} >> 1$

c.) If we have

19) $\frac{\frac{\rho r p}{K}}{1 + \frac{M}{K(M)}}$ rises with M

August 28, 1957

On the Mechanism of Enzyme Adaptation.

by Leo Szilard

The Enrico Fermi Institute for Nuclear Studies
The University of Chicago, Chicago, Illinois

It would appear that as a general rule -- with notable exceptions -- bacterial cells respond with the formation of adaptive enzyme when presented with a compound that they can metabolize, particularly if the compound resembles some degradation product of an amino acid or a nucleic acid residue. How does the bacterial cell accomplish this feat?

Large due to the work of Jacques Monod in Paris, as well as his co-workers, and more also through the work of Aaron Novick and Milton Weiner here in Chicago, much information has become available on the induced formation of the enzyme, β-galactosidase. This I have reason to believe is a rather atypical case, but it should be possible -- if one exercises caution -- to draw certain general conclusions from this one example which is, unfortunately, the only case where quantitative data are available.

This enzyme splits the disaccharide lactose into glucose and galactose, and if the bacteria are grown in the presence of a chemical analogue of lactose, the β-galactoside, TMG, which is not split by the enzyme, the enzyme level may be raised in the growing culture from a few molecules per bacterium to perhaps 10,000 molecules per bacterium. When the bacterium is used which requires an amino acid for its growth and when such bacteriia are grown in the Chemostat using this amino acid as a controlling growth factor, then the generation time, τ_{gen}, of the bacteria can be stretched at will up to perhaps tenfold. The enzyme level reached, when the bacteria are grown in the Chemostat (at a fixed internal concentration of TMG) is independent of the generation time, τ_{gen}.

Moreover on the basis of this mechanism, one should expect a more general law to hold, which says that the enzymatic composition of the bacterium does not change when we reduce the growth rate -- over a range of perhaps a factor of ten -- by slowing the rate of protein synthesis. Our formulae indicate the possibility of an exception to this rule in the case of mutants which contain an abnormally high level of an inducible enzyme in the absence of an inducer -- the so-called constitutive mutants. Perhaps one has to regard such mutants as pathological organisms, in which the normal regulation of the level of an enzyme has broken down.

This, of course, would be expected at very high TMG concentrations when the bacteria are fully induced, but it also true for lower inducer concentrations when the enzyme level is appreciable but not at its maximum possible value. This apparent "law of the growth rate independence of the enzyme level" furnishes, to my mind, an important clue to the general mechanism of enzyme induction.

How can inducer raise the enzyme level from a few molecules per cell to about 10,000 molecules per cell?

According to the notions here prewented, an enzyme is formed from amino acids which line up alongside a nucleic acid strand and then are joined by some chemical reaction chain into a polypeptide which, in turn, folds up into an enzyme. The nucleic acid strand, which imposes a specific amino acid sequence on the polypeptide, need not necessarily be the gene itself, although it must contain the same information as does the gene. We shall refer to it as the "paragene." For the sake of simplicity, we shall further assume that by definition the uninduced cell contains one paragene, for each kind of enzyme that the cell is capable of producing. If the enzyme level is raised several thousandfold, we can say either that the inducer increases the number of paragenes or else we can say that the number of paragenes for each specific enzyme remains unchanged, but the rate at which the enzyme is made is increased by the inducer. It is this latter view which we have adopted and we shall describe a mechanism which we believe may be responsible for the increase of the rate of enzyme production of the paragene. In a fully induced cell the rate of enzyme production is then determined by the time, $\tau(AA)$, that it takes to assemble the amino acids along the paragene and the time, $\tau(E)$, that it takes for the enzyme that is formed to evaporate from the paragene. Accordingly in the fully induced cell the number of enzymes is given by

$$(1) \quad N_{max} = \frac{\tau_{gen}}{\tau(E) + \tau(AA)}$$

For the sake of argument, I shall assume in this presentation that the uninduced cell contains one paragene for each kind of enzyme that the cell is capable of producing. If the enzyme level is raised several thousandfold, through the action of an inducer added to the medium, we can then either say that the inducer somehow increases the number of paragenes which are specific for this enzyme -- a possibility which we shall disregard -- or else we can say that the rate at which the enzyme is made by ~~the specific~~ this paragene is increased by the inducer and that ~~these paragenes do not increase in number.~~ no additional paragenes of this specificity are produced.

In the Chemostat, when the growth ~~rate~~ is ~~permitted~~ set to be ~~fast,~~ high, we ~~may write~~ have $\tau_{gen} \approx 4000$ sec, and we may estimate that we have $\tau(AA) \approx 1/2$ sec. and shall assume for the sake of argumentation consider

(2) $\tau(E) << \tau(AA)$

The value of $\tau(E)$ is independent of the growth rate in the Chemostat, and we shall assume for the sake of argument ~~that~~

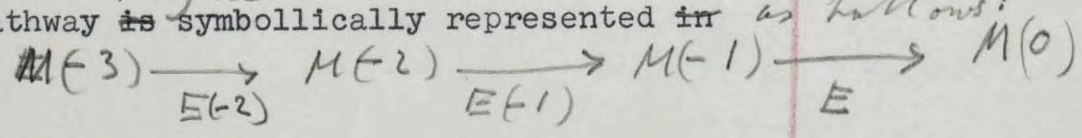
(3) $\tau(E) \approx \frac{1}{10}$ sec $<< \tau_{min}(A.A)$

With these values give according to (1) ~~Thus~~ for a fully induced cell growing in the Chemostat at a fast rate ~~, we obtain n = 10,000~~ for this one enzyme: $N = 10{,}000$ ~~enzyme~~ molecules/cell ~~specific~~

It is believed that ~~There~~ there might be about 10,000 different ~~paragenes~~ genes in a bacterium which contains about 10^{-13} grams of protein. If, on the average, the corresponding 10,000 different enzymes have a molecular weight of 100,000, ~~one finds that~~ then there must be, on the average, ~~there are~~ ______ molecules present in the cell of each kind of enzyme. ~~One may then say that, on the average, enzyme production by a paragene is repressed - ____ fold from the maximum rate at which the paragene could produce enzyme if it did produce the enzyme at the full rate. What prevents the paragene from producing its enzyme at the full rate?~~

Insert (2) Wrong theory.

~~At this point we shall abandon any further consideration of the enzyme, β-galactosidase, which I have reason to believe represents a particularly complicated case, and turn to enzymes~~ which lie on a straight bio- synthetic pathway leading from some precursors to amino acid ~~and leading beyond the amino acid~~ or on a pathway leading from the amino acid to its degradation product. Such a pathway ~~is~~ may be symbollically represented ~~in~~ as follows:



$$M(-3) \xrightarrow[E(-2)]{} M(-2) \xrightarrow[E(-1)]{} M(-1) \xrightarrow[E]{} M(0)$$

(3)

Insert 3 on page 3

One might attempt to account for the phenomenon of induction by saying that perhaps the enzyme formed along the paragene sticks ~~on~~ the paragene until a hypothetical inducer~~, which can~~ combine with the para-gene-enzyme complex~~, does so~~ and thereby somehow releases the ~~inducer~~ from the paragene. The paragene-enzyme complex -- so one ~~might further argue~~ -- cannot ~~synthize~~ synthetize ~~further enzyme~~ molesules until the enzyme leaves the paragene. It is easy to show that such a model would be in flagrant contradiction to the law of growth-rate independence on the level of the externally induced enzyme. ~~One can estimate that if, at a certain fast growth rate, the enzyme level~~ There are say 1,000 enzyme molecules per cell ~~when the bacteria are grown in the presence of~~ the inducer, and that when subsequently the growth of the bacteria is slowed in the Chemostat, say by a slow rate of protein synthesis lower than the concentration of tryptophan which the bacteria require as a growth factor, the enzyme level ought to drop to________.
~~This is in flagrant contradiction to all experience.~~

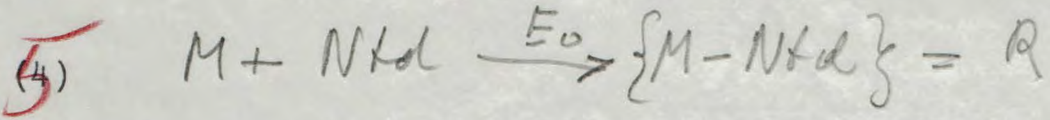
The metabolite, M(0), in this formula represents an amino acid and the ~~metabolites~~ metabolites, M(-1), M(-2), and M(-3) represent precursors of this amino acid. If the amino acid, M(0), is arginine, these precursors are in order: acetyl ornithin, ornithin, and citrulline. About a year ago Vogel reported that enzyme, E(-2), can be repressed in the bacterial ~~strain~~ in the W strain of coli with which he worked if the bacteria are grown in the presence of high arginine concentration. How can arginine repress the formation of such an enzyme?

We shall ~~presently~~ describe a mechanism, ~~depending on circumstances,~~ for a metabolite which can combine with the enzyme (just as presumably arginine can combine with the enzyme, acetyl ornithase) -- according to circumstances -- either to enhance or to repress the formation of the enzyme. The formation of the enzyme from amino acids along the paragene ~~and the time, τ(E), that it takes the enzyme to evaporate~~ are not per se influenced ~~by the metabolite~~. The time, τ(AA), that the process of the formation of the enzyme along the paragene takes depends only on the concentration of the amino acids in the cell, and if one particular amino acid controls the growth rate of the bacteria in the Chemostat, the time, τ(A), will increase proportionally to the generation time, τgen.

After the enzyme is formed on the paragene, the enzyme evaporates -- so we shall assume -- ~~when~~ an average time, τ(E), which is the same whether the enzyme sitting on the paragene is free or whether it is combined with the metabolite, M. But ~~when~~ the enzyme is combined with a repressor molecule which nails it to the paragene, then the enzyme cannot evaporate, and the paragene cannot synthetize additional enzyme molecules. According to the notions here presented, a repressor molecule is composed of two parts. It contains a moiety that ~~consists of~~ metabolite, M; and it contains a moiety M_{td}, which ~~can~~ combine with ~~certain nucleotides on~~ the paragene. The moiety, M_{td}, which must be able to combine with the paragene, might itself be a nucleotide, ~~but~~ for such a composite molecule to function as

as a repressor, the spatial configuration must be just right to permit a simultaneous complexing of the enzyme with the moiety, M, and of the paragene with the moiety, Ntd.

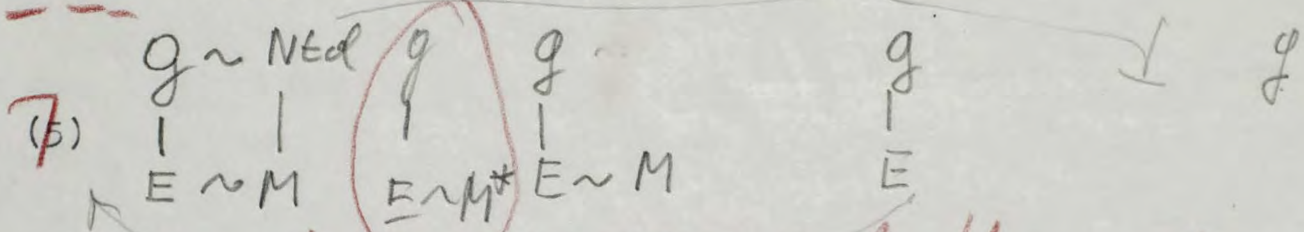
A repressor molecule, R, may or may not be formed by the cell from the metabolite, M, directly by means of the reaction



I assume that the cell may form arginine a repressor which contains arginine, and that this might be true for all amino acids. The enzyme which couples the amino acid to an Ntd moiety and thus forms a repressor, we may designate as a coupled enzyme, E^*.

If the repressor, R, is combined with the paragene-enzyme complex, it will evaporate after an average sitting time of $\tau(R)$, and when it does so the enzyme remains on the paragene -- so we shall assume.

Accordingly, the paragene-enzyme complex can be present in three forms which may be symbolically written as follows:



For the rate of formation, s, of the enzyme by one paragene, we may write

$$(6)\quad s = q\,\frac{1-p}{\tau(E)}$$

~~where~~ q denotes the fraction of the paragene on which there is sitting a completed enzyme molecule (whether uncombined or combined with the metabolite, M, or combined with the repressor, R), and we may write

(~~7~~ 6) 8 $$q = 1 - s\,\tau(AA)$$

Page

~~and where~~ and p denotes that fraction of the enzyme molecules sitting on the paragene which are nailed down by the repressor, R. Accordingly, we have for p

(8) $$p = \frac{R/K(R)}{1 + M/K(M) + R/K(R)}$$

or

write $\frac{1}{1-p}$

(9) $$1 - p = \frac{1 + M/K(M)}{1 + M/K(M) + R/K(R)}$$

where K(M) is the Michaelis constant of the enzyme for the metabolite M and ~~where~~ K(R) is the Michaelis constant of the paragene-enzyme complex for the repressor, R. From (6) and (7), we obtain for s the rate of enzyme production of the paragene

replace by 19 type

(10) $$s = \frac{(1-p)}{\tau(E) + (1-p)\,\tau(AA)}$$

and for the number of molecules, N, of this enzyme maintained by one paragene in the bacterial cell, ~~during growth~~ during growth we obtain

replace by 19 type

(11) $$N = s\,\tau_{gen} = \frac{(1-p)\,\tau_{gen}}{\tau(E) + (1-p)\,\tau(AA)}$$

and put in $\frac{1}{1-p}$ explicitly from 8

Under ordinary conditions when we have, as stated above, $\tau(AA)$ = 1/2 sec.; τ_{gen} = 4,000 seconds, and $\tau(E)$ = 1/10th second. If the enzyme is not repressed so that we have to write p = 0, equation (11) gives for the number of enzyme molecules N = 10,000 per cell.

If we have the enzyme production repressed by at least a factor of 10 -- and in general this will be true of almost all enzymes --, then we have

$$\tau(E) >> (1-p)\,\tau(AA) \quad (12)$$

and

$$1-p << 1$$

Let us now consider the case of a metabolite, M(0) -- like arginine -- from which a repressor, R(0) can be made through reaction (4). And let us in particular consider first the case where the concentration of a repressor, R(0) is proportional to the concentration of the metabolite, M(0) in which case we may write

$$\frac{R(0)}{K(R)} = C \times \frac{M(0)}{K(M)} \quad (13)$$

where C is independent of [M(0)] and larger than 1.

As equations (9) shows, if in these circumstances we lower the concentration of the metabolite, M the factor (1-p) increases from an initially small value and approaches 1, according to (11) the enzyme level, N, will and approach the value N_∞ given in (1)

(3) If the arginine concentration inside the bacterium is lowered below the value which it has when the bacterium grows in minimal medium in the absence of arginine, then, as may be seen from equation (9), (1-p) is approaching 1 as the arginine concentration is approaching 0, and according to equation (11) the enzyme level must then approach the value, N_{max}, given in equation (1), which is about 10,000.

If, on the contrary, we raise the arginine concentration by adding some arginine to the medium in which the bacteria grow, then the change in the enzyme level will depend on whether in the bacteria which grow in the absence of arginine we have

$$(14) \qquad \frac{M(0)}{K(m_0)} \ll 1$$

or whether we have

$$(15) \qquad 1-p \Rightarrow \frac{1}{1+C} \quad \text{for } M_0 \Rightarrow \infty$$

If equation (14) holds, raising the arginine concentration will lower the enzyme until the enzyme level reaches and equals__________. On the other hand, if equation (15) holds, then raising the arginine concentration will no longer cause any appreciable change in the enzyme level.

In this case, adding more and more arginine to the medium will lower the value of 1-p which can ultimately be pushed down to

(15) $$1-p = \frac{1}{1+C}$$ giving from 11

(16) $$N = \frac{\tau_{gen}}{(1+C)\,\tau(E) + \tau(AA)}$$

On the other hand if if, in the bacteria growing in the absence of arginine in minimal medium, we have

(17) $$\frac{M(0)}{K\{M(0)\}} >> 1$$

then adding arginine to the medium will ~~not~~ no longer appreciably ~~change~~ lower the value of 1-p and the enzyme level will not appreciably be affected by the addition of arginine.

Clearly a prediction of this kind can be subjected to experimental test, and experimental evidence already available ~~and~~ though perhaps published might bear out the contentions of this theory. ¶ ~~As may be seen from equation (11), as long as enzyme~~ is still sufficiently repressed; i.e. as long as we have

(18)

An ~~increase of 1-p will,~~ according to equation (11), raise the enzyme level of the bacteria, and this means, as one may see from equation (9), that any metabolite, M^*, that combines with the enzyme and that does not form a repressor will increase 1-p, and therefore raise the enzyme level in the growing bacterial culture.

And even if the metabolite does form a repressor, increasing the concentration of the metabolite, M^*, will raise 1-p, and therefore raise the level of the enzyme in the growing culture, provided only that -- counting all the different repressors, R-I, which may be present -- the expression summa   rises more slowly than the concentration of the

Only the case of an enhancement of enzyme production, which we have so far discussed within the framework of this theory, was based on the reduction of the concentration of a repressor. Thus a lowering of the arginine concentration raised the level of the enzyme which was repressed by Ntd coupled arginine. But is there such a thing as an inducer which will enhance the formation of the production of an enzyme directly rather than through inducing the concentration of a repressor?

Clearly, if we have a chemical analogue of the metabolite, M, which can combine with the enzyme, just as does the metabolite, M, but which the cell cannot be coupled with a Tdn moiety to form a repressor, such substances, M^*, could be regarded as a genuine direct inducer. In the presence of M^*, we have xxx an analogy to equation (9)

$$(18) \qquad 1-p = \frac{1+\frac{M}{K(M)}+\frac{M^*}{K(M^*)}}{1+\frac{M}{K(M)}+\frac{M^*}{K(M^*)}+\frac{R}{K(R)}}$$

and it may be seen that by increasing the concentration, M^*, we may increase 1-p, and as long as inequality (12) holds, it follows from equation (11) that the number of enzyme molecules per cell maintained in the growing culture must go up when the concentration of M^* is raised.

The precursor of a metabolite in a biosynthetic pathway which leads to an amino acid, while it certainly can combine with the enzyme which carries it one step forward in the biosynthetic pathway towards the amino acid, may -- generally speaking -- not be an inducer because by raising its concentration, the concentration of R(0) may also be raised.

but it might be converted into the repressor R and might therefore not be an inducer.

The situation may be quite different in this respect, as we shall discuss later, in biosynthetic pathways which lead from amino acids, such as, for instance, tryptophan through a number of degradation products. We may now ask whether (to what extent) our theory can account for the law of the growth-rate independence of the enzyme level, which was mentioned earlier, in the case where (when) the enzyme is induced by maintaining a fixed concentration of an inducer, M^*, in the nutrient medium, when we change the generation time, τgen, of the bacteria growing in the Chemostat.

The term τ(E) is negligible compared to the term τ(AA) and of the three terms

In order to say (see) this, we write equation (11) in the form

$$(19) \quad N = \frac{\tau(\text{gen})}{\tau(E) \times \frac{1}{1-p} + \tau(AA)}$$

and for $1/1-p$ we write, from equation (18)

$$(20) \quad \frac{1}{1-p} = 1 + \frac{R/K(R)}{1 + M/K(M) + \frac{M^*}{K(M^*)}} \approx 1 + \frac{R}{M^*}\,\frac{K(M)}{K(R)}$$

If we then assume that we can include (neglect) τ(E) compared to τ(AA) (in comparison) and if we further (now) assume that the repressor, R, is made by the bacterium at a constant rate so that its steady state concentration in the growing culture becomes proportional to τgen so that we may write: τgen/τ(0)gen, then we may write in place of equation (19)

we obtain from (19) and (20):

$$(21) \quad \frac{\tau_{gen}(1)}{\tau_{gen}(2)} = \frac{R(1)}{R(2)}$$

$$(21) \quad N = \frac{\tau\,\text{gen}}{\tau(E)\,\frac{R}{M^*}\,\frac{K(M)}{K(R)} + \tau(AA)}$$

which shows that the enzyme level maintained in a growing culture at a fixed external inducer concentration, M^*, is independent of the generation time. W.Z.B.W.

If we now assume that then all terms in 21 are proportional to τgen and

A direct inducer acts as an inducer because it competes with the repressor for the paragene-enzyme complex. It follows from this that while a direct inducer can enhance the formation of the enzyme, it can raise the enzyme level in the growing culture in the steady state at best proportional to its concentration.

A chemical analogue of a metabolite which combines with the enzyme can, however, also act as an indirect inducer, for instance, by inhibiting the formation of a repressor. In such a case the enzyme level in the steady state can go up faster than linearly with the inducer concentration.

We must now turn our attention to the metabolic pathways which lead from an amino acid step by step through a number of degradation products. An example of such a pathway is the degradation from tryptophan along the pathway which leads to______. According to Roger Stanier, this pathway leads through X enzymes, y of which can be induced by the metabolite which is carried by the enzyme one step further along the biosynthetic pathway. How can we understand that in the case of such a pathway the precursor so frequently induces the enzyme?

~~It is conceivable that in such cases, we are dealing with two parallel pathways which may be symbolically written as follows~~

~~(22)~~

In order to explain this, we might assume that the repressor which fits the enzyme-template complex for any given enzyme will do so mainly if it is composed ot Ntd moiety which might very well be the same for the whole chain of the   which is produced by the enzyme rather than its precursor. We have no model to justify this assumption, and the belief that it is so can therefore be based only on the experimental fact of the teleological argument that this would give a reasonable regulation. We could then explain the fact that all these metabolites induce the proper enzyme by simply saying that these metabolites are not readily converted into the corresponding repressor, and therefore act as an inducer of the enzyme simply because they may be expected to be fairly tightly bound by the enzyme which carries them through the next step along the biosynthetic pathway. The compound into which they are thus transformed by the enzyme may also combine quite well with the enzyme, and therefore we should expect, at least on occasions, the induction of the enzyme not only by the precursor of a given metabolite but also by the metabolite itself. Some cases of such back induction appear to have been observed in fact. One ought to expect that the level of an enzyme along such a metabolic pathway rises no faster than linearly with the concentration of the metabolite that serves as an inducer present in the medium. It is, however, conceivable that such metabolites act not only as a direct inducer but also as an indirect inducer, and we shall now illustrate this possibility by presenting a rather interesting scheme which might conceivably hold. According to this scheme, we are dealing with two parallel pathways which may be written symbolically as follows:

(22)

Here N(+1) is a Rrepressor of the enzyme E(N). The enzyme E(N) transforms both R(N) into R(N+1) and also M(N) into M(N+1). Only the metabolite M(0) is coupled directly by the coupling enzyme E(0) to Ntd and forms by direct coupling the repressor, R(0), and the other repressors along the metabolic pathway are supposed to be made not directly from the metabolite which is carried by the enzyme one step further along the metabolic pathway, but are made by the enzyme forme from another repressor.

If this were in fact the scheme of things along such metabolic pathways, then clearly any metabolite M(N) along such a pathway would of necessity induce the enzyme, E(N), because it combines with this enzyme and is not transformed into a repressor, and hence according to our definition any metabolite, M(N), would be a direct inducer.

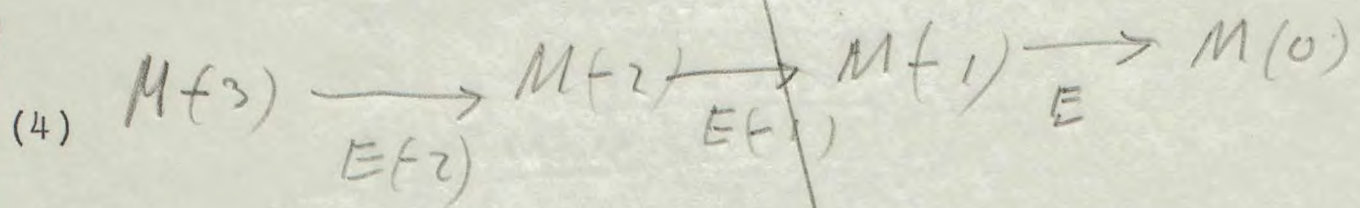
In the scheme here presented the metabolite would not only act as a direct inducer but also as an indirect inducer because it would compete with the homologous repressor for the enzyme, E(N). As a result of this, the metabolite M(N-1) will compete for the enzyme, E(N) and therefore reduce the concentration of the repressor, R. If this takes place, the metabolite, M(N), acting as an inducer will raise the level of the enzyme, E(N), and the enzyme level will rise faster than linearly with the concentration of the metabolite, M(N).

By slowing the flow from the point, N, on along the second metabolic pathway, and thereby reducing the concentration of all subsequent repressors, all the enzymes along the whole pathway from the nth enzyme on ought to be enhanced. Whether it is possible experimentally to distinguish between these phenomena in the scheme discuss and their sequential induction represented by enhancement of these enzymes by the metabolite, M(N) due to the production of the metabolites N(+1), N(+2) and

N(+3) remains to be seen. The lowering of growth-rate independence on the enzyme levels will hold along such a metabolic pathway, provided that the enzymes are not saturated by the metabolites, and provided that the flow along the pathway is slow so that $M(0)/\tau$ is greater than the rate at which M(0) is carried through the next biochemical step by the enzyme E(1).

concentration of tryptophan that the bacteria require as a growth factor.
This is in flagrant contradiction to all experience.

At this point we shall turn our attention from the induction of the enzyme, β-galactosidase, to the regulation of the level of enzymes which lie on a straight biosynthetic pathway leading from some precursors to amino acid or on a pathway leading from the amino acid to its degradation product. Such a pathway may be symbolically represented as follows:

(4)


The metabolite, M(0), in this formula represents an amino acid and the metabolites, M(-3), M(-2), and M(-1) represent precursors of this amino acid. If the amino acid, M(0), is arginine, these precursors are in order: acetyl ornithine, ornithine, and citrulline.

About a year ago Vogel reported that the enzyme, acetyl ornithine -- in our notation E(-2) -- can be repressed in the W strain of coli if the bacteria are grown in the presence of a high arginine concentration. How can arginine repress the formation of such an enzyme?

We shall now describe a mechanism by means of which a metabolite which can combine with an enzyme -- just as presumably arginine can combine with the enzyme acetyl ornithase -- may according to circumstances either enhance or repress the formation of that enzyme.

Amino acids are assembled in the proper sequence along the paragene and are joined into a polypeptide through some chemical reaction chain. This polypeptide, in turn, folds up into the enzyme. On the average this

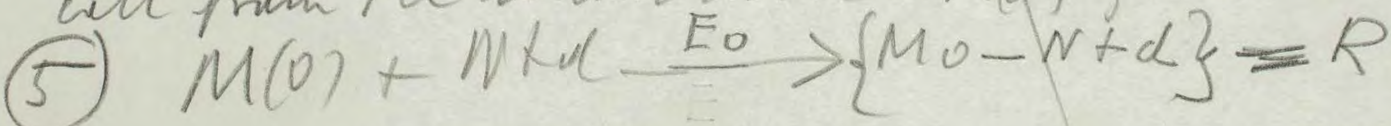
process takes a time, τ (AA). If the bacterium which requires tryptophan is grown in the Chemostat, the time, τ (AA), can be stretched perhaps tenfold by lowering the tryptophan concentration in the growth tube.

~~Let us assume that~~ there is present in the cell a metabolite, M(0), such as arginine which can combine with ~~the~~ enzyme, and let ~~us~~ further assume that the cell is capable of producing a molecule, R, which we shall call a repressor and which is composed of two parts: It contains a moiety that can combine with the enzyme, such as the metabolite, M(0), and it contains a moiety, Ntd, which can combine with a certain part of the paragene -- perhaps a group of nucleotides. The moiety, Ntd, might perhaps itself be a nucleotide, If this repressor molecule fits onto the paragene-enzyme complex in such a manner that the moiety, M, can combine with the enzyme at the same time as the moiety, Ntd, combines with the paragene, then -- so we shall assume -- the repressor covers the paragene-enzyme complex ~~in such a manner that~~ the enzyme cannot evaporate from the paragene ~~until the~~ repressor ~~evaporates from~~ the paragene-enzyme complex. ~~We shall designate~~ the average time that it takes for such a repressor to evaporate as τ (R).

We shall, for the sake of completeness, further assume that some chemical analogue, M*, of the metabolite, M(0), may be added to the medium in which the bacteria grow. ~~It is assumed that M*~~ can combine with enzyme but does not get incorporated into a repressor molecule. ~~The~~ enzyme which has been formed will evaporate off the paragene with ~~an~~ average lifetime of τ (E) whether ~~or not~~ the enzyme is combined with ~~either~~ the metabolite, M, or the metabolite, M*, ~~provided only the paragene-enzyme complex is not combined with the repressor.~~

~~We may now~~ explain Vogel's observation ~~if~~ we assume ~~that~~ ~~the cell may form such a repressor from arginine.~~ ~~The enzyme, E(0), which~~

A repressor molecule R may be formed in the cell from the metabolite M(0) by the reaction



(5) $M(0) + Ntd \xrightarrow{E_0} \{M_0 - Ntd\} \equiv R$

may couple an amino acid (such as arginine) to an Ntd moiety to form a specific repressor for some of the enzymes which lie in the biosynthetic pathway that leads to the formation of arginine, we may designate as the coupling enzyme for arginine. There might be other such specific coupling enzymes present in the cell for other amino acids. The cell may contain such coupling enzymes, E(0), for other amino acids, and each coupling enzyme, E(0), might be specific for one amino acid.

We shall now attempt to compute on the basis of this model the rate, s, at which an enzyme is synthetized by a paragene in the presence of the metabolite, M, the inducer, M*, and the repressor, R. If we assume

(5)

we will obtain a simple formula for the rate of enzyme formation and the result will be consistent with the established facts if we assume

(6) $\tau(M)$ as well as $\tau(M^*) \ll \tau(E) \ll \tau(AA)$ as well as $\tau(R)$

Accordingly the computation here presented will assume these inequalities.

For the rate of the formation, s, of the enzyme by one paragene, we may write

(7)

where q denotes the fraction of the paragenes on which there is sitting a completed enzyme molecule (whether uncombined or combined with the metabolite, M, or combined with the repressor, R), and we may write

(8)

and p denotes that fraction of the enzyme molecules sitting on the paragene which are nailed down by the repressor, R.

As stated before, the metabolite, M, the inducer, M*, and the repressor, R, can all reversibly combine with the paragene-enzyme complex, and the respective Michaelis constants I shall designate by: K(M); K(M*), and K(R). Accordingly, the paragene-enzyme complex will be present in four different forms which are symbolically represented as follows:

(9)

The metabolite M

-- by combining with the enzyme molecule which sits on the paragene -- also protects the paragene-enzyme complex against being covered by the repressor. However, this effect of the metabolite, M, may or may not be counterbalanced by virtue of the fact that the cell can transform the metabolite, M, into a repressor, and the metabolite, M, may therefore raise the concentration of the repressor molecules in the cell. Accordingly, the metabolite, M, will, depending on the circumstances, either enhance the formation of the enzyme or repress it, in contradistinction to the chemical analogue, M*, which cannot be transformed into a repressor molecule and must therefore always enhance enzyme production.

What kind of molecule must the repressor molecule be in order to be able to prevent the evaporation from the enzyme molecule which has been formed from the paragene? We assume that such a repressor molecule contains a moiety that can combine with the enzyme, such as a metabolite, M(O), and it must contain a moiety, Ntd, which can combine with a certain part of the paragene -- perhaps a group of nucleotides on the paragene. It is conceivable that the moiety, Ntd, might itself be a nucleotide; hence the designation, Ntd. For such a molecule, R, to function as a repressor, it is necessary that the repressor "fit" the paragene-enzyme complex in the sense that the moiety, M, must be able to hold on to the enzyme at the same time the moiety, Ntd, holds on to the paragene. This imposes a very specific condition on the spatial configuration of the repressor, R.

Such a repressor molecule, R, may be formed in the cell from the metabolite, M, by the reaction

(6)

The enzyme, E*, may be called a coupling enzyme and such a coupling enzyme, as well as the moiety, Ntd, might be specific for each metabolite, M, although a number of metabolites along the same biochemical pathway might be able to do with the same coupling enzyme of the same Ntd moiety.

The combination of the metabolite, M, and the inducer, M*, with the enzyme is reversible and so is the combination of the repressor, R, with the paragene-enzyme complex, and we may write for the Michaelis constants respectively K(M), K(M*), and K(R). Similarly we may write for the average time which it takes for M, M*, and R to evaporate τ(M), τ(M*), and τ(R).

The model here adopted assumes that when the repressor evaporates from the paragene-enzyme complex it leaves the enzyme on the paragene.

The concentration of M, M*, and R, that are maintained in the cell, we shall designate by [M], [M*], and [R].

We shall now compute the number of enzyme molecules maintained per bacterial cell in a growing bacterial culture on the assumption that we have

THIRD VERSION May 9, 1957

Suppressor Mutations, Enzyme Induction, and Antibody Formation

By Leo Szilard

The Enrico Fermi Institute for Nuclear Studies
The University of Chicago, Chicago, Illinois

INTRODUCTION:

I shall attempt to present in this paper the groundwork for a theory that, I hope, may in time permit us to explain in detail all the known facts concerning adaptive enzyme formation and antibody formation. According to this theory, as we shall see, the distinction between induced and non-induced enzymes disappears. In principle, it should be possible to synthesize certain chemical analogues for the great majority of the metabolites which will act as an inducer for the enzyme that produces that metabolite. The metabolite itself, however, if it is a precursor of a purine, pyrimidine or amino acid, for instance, would not be expected to be an inducer. The problem with which we have to deal is deeper than to find an explanation for the mechanism through which an inducer can enhance the rate of production of an enzyme.

There is a vast area of compounds which are potentially oxidizable by a bacterium such as P fluorescence. R. Y. Stanier estimates the number at 50 and estimates that at least 200 inducible enzymes must participate in their dissimilation. Why are all these enzymes present in the bacteria. Even if there has been some use for them at some time, why would they persist - as I am convinced they would - if we cultivated the bacteria for a long period in the absence of any of the substrates of

these enzymes? Why would these enzymes not be gradually lost by mutations during such a long period of cultivation while there is no selection operating in their favor? I believe these questions are answerable and that we must abandon the untenable belief that these enzymatic potentialities are stored away in the genome in order to be simply and rapidly activated through sequential induction when a suitable substrate fixes their environment. I believe that these enzymes have a reason during evolution through suppression mutations, and that they persist because they play a role in the complex regulatory system -- to be described later -- which determines the rate of production of most bacterial enzymes.

According to the theory here presented, these enzymes are all present at low levels in bacteria which grow in minimal medium, and they persist because, if one of them disappears through a mutation, the levels of the different enzymes in the bacterium would be out of balance. Support for this view, as we shall see later, comes from the experiments of Yanowsky and co-workers, who found that mutations in the Neurospera which lower the rate of production of the enzyme, tryptophane synthesase, can be restored by suppressor mutations which occur in different genes, but that the different mutations often all lie within the same conventional chain require different suppressors to restore the ability of the cell to form tryptophane synthesase in adequate amounts. They also found that all those mutants for which a suppressor can be found form an immunological analogue of the enzyme, tryptophane synthesase, that lacks enzymatic activity. Once the connection between this phenomena and the ability of the bacterium, like P-fluorescence, to dissimilate a vast array of oxidizable compounds with the help of inducible enzymes, the phenomenon of drug-tolerance, drug addiction, and antibody formation becomes understandable also.

The basic concepts, upon which the theory here presented is based, are as follows: enzymes are produced by some templates, and each specific template produces a corresponding enzyme. The enzyme-template-complexes in bacteria can dissociate off enzyme at a rate which is about 10^4 times as high as the rate at which ~~on the average~~ ~~the~~ templates produce enzyme in ~~the rapidly growing bacterium~~. In general, enzyme production is repressed by a reversible combination of the enzyme-template-complex with the repressor. While the enzyme-template-complex is thus covered by the repressor, the template does not dissociate off enzyme. What are these repressors?

the basic scheme of repressors

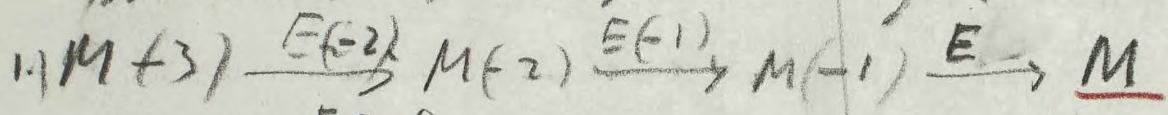
Amino acid, purines and pyrimidines are essential building blocks from which polymers, such as proteins and nucleic acids are built, and we shall ~~designate~~ metabolites of this sort as metabolites of 0 order. (In a sense, hexose residues from which polysaccharides are built are similar building blocks, but in one essential respect they differ from amino acids and nucleotides we shall avoid designating them as zero order metabolites. The M-R complex ~~is presumably the vehicle~~ through which the amino acids and purines are built into the larger structures of proteins and nucleic acids.

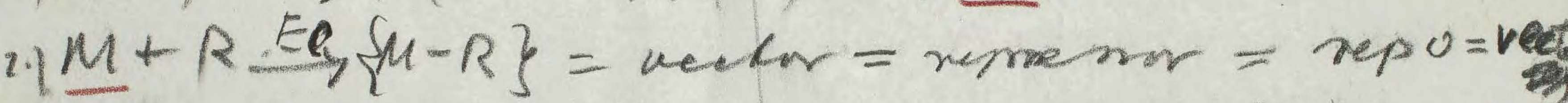
~~The zero order metabolites, M, are carried further along, so we shall assume, two parallel metabolic pathways, presumably both handled by the same enzymes, E(1), E(2), and E(3). Along the one pathway moves the metabolite complex with the carrier, R, and the metabolite is degraded or modified in each step, while the carrier, R, remains unchanged. Along the parallel line the metabolite itself is modified or degraded by the same enzymes. We shall refer to the metabolite, M(n), as a postcursor n steps removed from the M, whereas the precursors n steps removed from the metabolite, M, will be designated by M(-n).~~

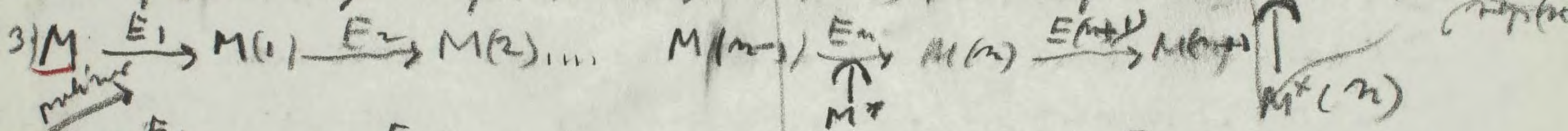
We assume that specific coupling enzymes, E_0, are present in the cell which couples the specific metabolite, M, to the non-specific carrier.

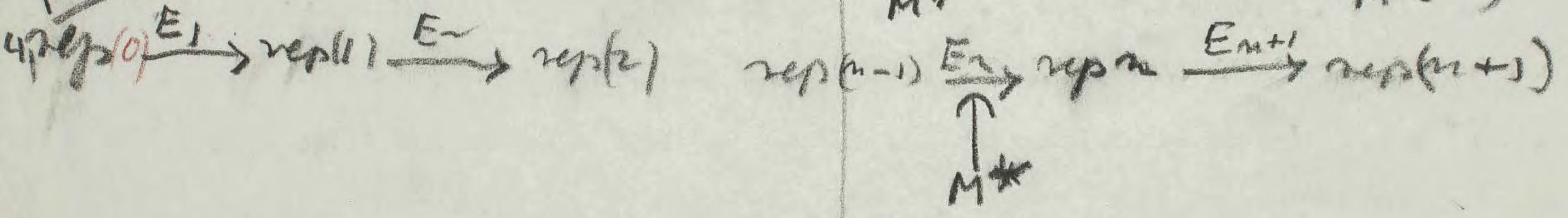
We shall, however, not assume that the coupling enzyme, E_0, can couple with R any of the postcursors, M(n) of the metabolite, M. We shall write rep(M) as an abbreviation for M-R and its postcursors n steps removed, in which M is degraded or modified, we shall designate by rep(n).

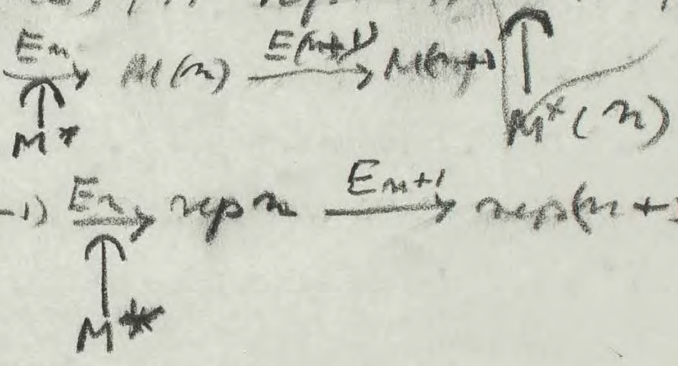
~~The Repressor of 0 order~~







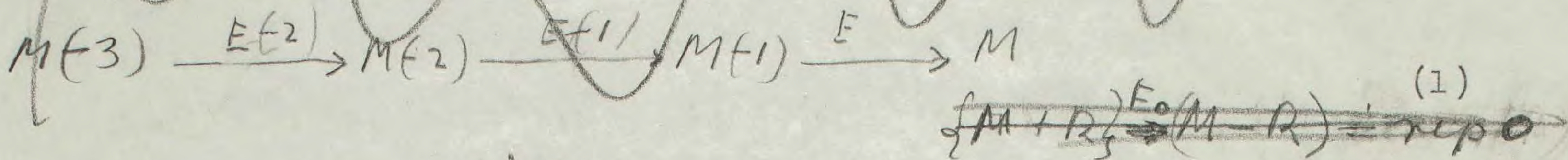




Metabolic pathway No. 1 leads from a precursor, n steps removed from M(-n), to the metabolite, M. In metabolic step 2 the metabolite, M, is coupled to the carrier, R, to yield the vector through which the metabolite, M, is carried into a polymer such as proteins, nucleic acids, polysaccharides, etc. This vector also functions as a repressor of enzyme induction for the coupling enzyme itself, and presumably for earlier enzymes called E(-n), one or a few steps removed. The biosynthetic pathway 3 leads from the metabolite, M, to degradation products and otherwise modified forms of this metabolite, to which we shall refer as postcursors in contradistinction to the metabolites which are precursors of the metabolite, M, which is n steps removed from the enzyme, E, is designated by E(n). We assume that the same enzymes, E(n), which are active in this metabolic pathway are also active in the metabolic pathway which leads from M-R to M(n)-R or from rep to rep(n). The characteristic features of this scheme is the absence of enzymes which would couple the postcursor, M(n) to the carrier, R. Thus, the only way rep(n) is produced in the cell is from rep through mediation of the enzymes E(1) to E(2).

The Repressors

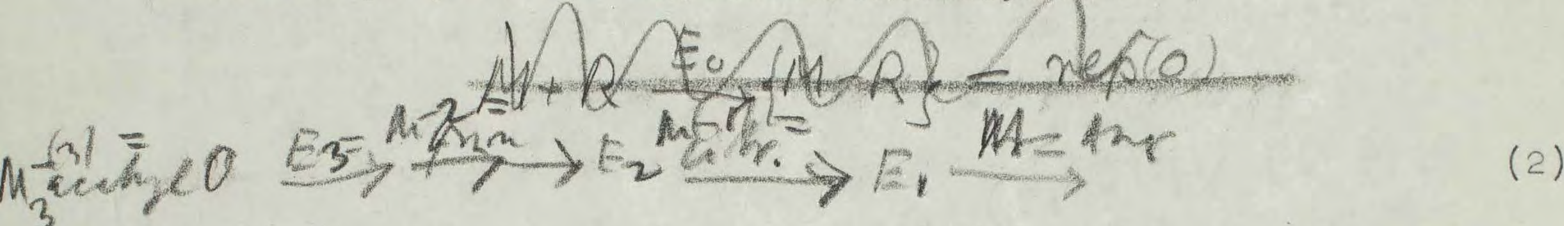
The metabolic pathway which leads to a metabolite of 0 order, M, and which may (this may for example) be an amino acid; for instance, arginine may be written as follows



(1)

For the sake of communication, we are disregarding throughout this paper metabolic pathways which are branched.

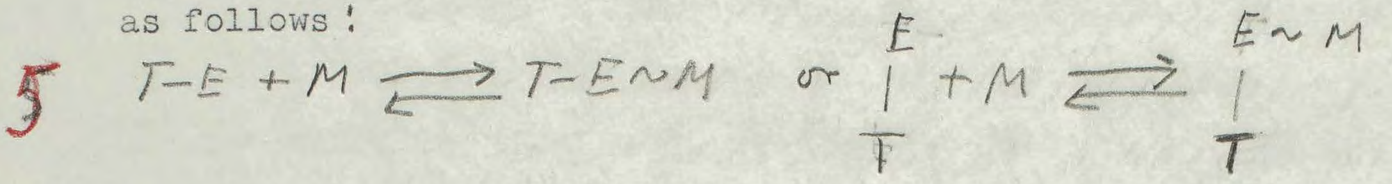
In the case of several amino acids; for instance, arginine, or tryptophane, many of the precursors are known and most of the enzymes have been isolated. In the case of arginine, for instance, we have M(-1) = citrulin; M(-2) = ornithin; M(-3) = acetyl ornithin. The enzyme, E_0, is a specific coupling enzyme which couples the specific metabolite, M, to the unspecific carrier, R.



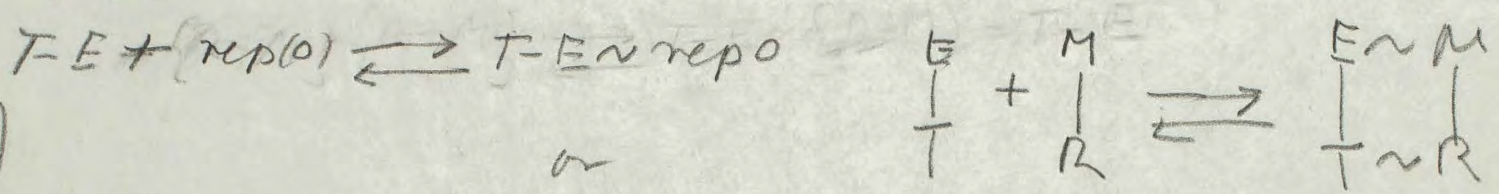
(2)

The complex, M-R, is presumably the vehicle by means of which the metabolite is transferred to a larger structure to form a protein or nucleic acid or (mutatis mutandis, a polysaccharide), but what is important for our purposes here is not the fact that M-R is a vehicle of transfer but that it is a repressor for the production of the enzyme, E, and conceivably also for the preceding E(-1), E(-2), and E(-3). It is a basic assumption of the theory here presented that the enzyme-template-complex can combine reversibly, both with the metabolite, M (as well as

certain of its chemical analogues, and ~~among them some of~~ such as, for instance, its precursors) and that the complex so formed does not hinder the enzyme-template-complex from dissociating off enzyme at a rapid rate. We shall further assume that the carrier-coupled metabolite, M; i.e. the repressor, M-R, or rep(o) ~~for which we shall use the abbreviated designation of rep(M)~~, can also reversibly combine with the enzyme-template-complex. In contradistinction to the enzyme-template-complex, which is ~~complexed~~ combined with the metabolite, M, the enzyme-template-complex, which is covered by the repressor, rep(M), <u>cannot</u> dissociate off the enzyme. We have thus two equilibria and two equilibrium constants, K(M) and K(rep), which are symbolically represented as follows:



and



The two equilibrium constants involved may be designated as K(M) and K(rep)

Protection

Accordingly the fraction of the templates which is free to dissociate off enzyme at the full rate is given by

$$(7)\quad f = \frac{1 + \frac{M}{K(M)}}{1 + \frac{M}{K(M)} + \frac{rep}{K(rep)}}$$

The enzyme E is ... produced at a rate which is proportional to f

$$S(E) = \text{Const}\, f$$

Apart from some very exceptional cases, we may expect ~~to have~~ that ~~[illegible]~~ living in bacteria (as well as other microorganisms) ~~which grow in~~ growing minimal medium ~~and un~~ ~~inequalities~~ ~~which may be written as follows~~ the following to hold

$$\frac{\mu}{K(m)} \ll \frac{rep(0)}{K_{rep}} \gg 1 \qquad (1)$$

? Use ?

If the equilibrium constant, K_{rep}, for this reaction is small; i.e. if ΔH for the formation of the covered complex is large, then the equilibrium is shifted most of the way toward the inactive form of the enzyme-template-complex, and enzyme production is strongly repressed. It is our notion that on the average enzyme production by a template will be a factor of about 10^4 below the full rate at which the uncovered template would produce enzyme. To accomplish this each repressor might have to be present at a concentration of perhaps 100 gamma per liter, and if we assume 10,000 different, specific templates and a repressor for each, the total repressor concentration might amount to one gram per liter. How does the repressor work ... According to the notion here presented, the repressor might affix the head of the enzyme to the head of the template. Perhaps the head of the enzyme, E, combines with the metabolite, M, and the carrier, R, (a nucleotide?) combines with the head of the template, as is somewhat vaguely indicated by the symbolism used above.

We presume ~~It is conceivable~~ that even though the head of the enzyme is affixed to the head of the template, the tail of the enzyme can detach itself occasionally (and might then each time be resynthesized inside the template). Thus it seems possible that debris which can immunologically cross-react with the enzyme will be formed at a certain - rather low - rate, while enzyme production is practically completely repressed. But even if the repression is lifted (by growing the bacterium in the presence of an inducer that enhances the production of the enzyme), the debris may be still produced although it might perhaps be produced at half the normal rate. We may presume that even in a highly induced cell at least half of the time the enzyme ~~lives~~ lies alongside of the template. I believe ~~believe~~ that in fact the immunological analogues of the enzyme tryptophane

synthetase described by Yanowsky (which he calls CRM) and the immunological analogue of the enzyme β-galactosidase described by Monod and his co-workers (which they call P_Z) are just the kind of debris ~~for which this theory can easily account.~~ that I have postulated

What is a real inducer

Keeping this in mind, we may now ask what ~~we may expect~~ will happen to the rate of production of enzyme E (or some of the preceding enzymes, E(n), which are ~~only~~ one or a few steps removed) when we add to the minimal medium in which the bacterium is growing a certain quantity of the metabolite, M. Pour fixer les idées, let us assume that the metabolite, M, is a particular amino acid, for instance, arginine. In general the concentration of the vector, rep(o) of this particular amino acid will not be the limiting factor for the rate of growth of a given bacterium in minimal medium, and therefore the rate at which the vector, rep, disappears (by transferring M to protein) may be expected to be independent of the concentration of rep. In such circumstances, when we add a certain quantity of metabolite, M, to the medium and thus raise the internal concentration of this metabolite within the bacterium, ~~we may expect~~ the concentration of rep ~~to~~ increase proportionately to the concentration of the metabolite, M, ~~in the bacterium~~.

In such a case, equation No. 7 shows ~~that~~ the rate of enzyme production will decrease ~~universally~~ with increasing concentrations of M within the bacterium (If $\frac{M^*}{K_M} \ll 1$) or else it will remain unchanged (If $\frac{M^*}{K_M} \gg 1$)

We shall define as a ~~pure~~ inducer a chemical analogue, M*, of the metabolite, M, which ~~is capable of reversible formation~~ ~~complex to which~~ the coupling enzyme can ~~be~~ attached ~~to~~ the carrier so that ~~it~~ is not ~~built~~ into a repressor, ~~andmoreover~~

~~Page 7 - continued 2~~

A direct inducer ~~which does not in any other way (hereby inhibiting the coupling enzyme, E_o) affect~~ leaves unchanged the concentration of rep. We may write in this case

$$f = \frac{1 + \frac{M^*}{K(M^*)} + \frac{M}{K(M)}}{1 + \frac{M}{K_M} + \frac{M^*}{K(M^*)} + \frac{rep^o}{K_{rep^o}}} \qquad (2)$$

~~and the compound~~ M* will enhance the rate of production of the enzyme ~~because it~~ leaves, as we have assumed, the concentration of rep unchanged. It will further, if it inhibits the coupling enzyme, E_o, ~~and~~

rate at which the enzyme-template-complex does. The inducer M* protects the enzyme-template-complex from being covered by the repressor. If the equilibrium constant, K_{M^*}, is low enough (i.e. if the binding energy, ΔH,

is high enough), then repression is relieved and the ~~inducer~~ enzyme is produced at a high rate even at moderately low concentrations of the inducer, M*.

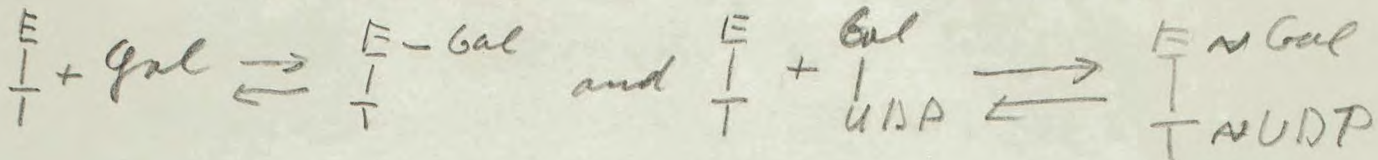
It is clear from any equations that the rate of enzyme production cannot rise any faster than linearly with the concentration of the direct inducer within the cell.

Indirect inducers A chemical analogue of M* of M may, however, also enhance the rate of production of the enzyme by competing for the enzyme which produces the repressor for the substrate of that enzyme. An inhibition by M* of the coupling enzyme, E_o, would be an example of this type of action. A chemical metabolite which does this we shall call an indirect inducer. That indirect inducers, i.e. substances which enhance the formation of an enzyme by inhibiting an enzyme that produces an "inhibitor" must exist was first postulated by Werner Maas (oral communication, April 26th, 1957). The view which he exposed at that time; viz. that all inducers are indirect inducers was a challenge to which the considerations presented in this paper represent a response. One must indeed invoke the existence of indirect inducers postulated by Maas in order to explain those cases where enzyme production is enhanced by an inducer and rises more rapidly than linearly with the inducer concentration within the cell. The induction of the enzyme, β-galactosidase by thiomethyl galactoside (TMG) one of the best studied systems is one case in point such indirect in addition to direct induction must be invoked.

What is an introduction ? T.P.G.

an inducer only exceptionally, or in the case of a certain class of metabolic pathways. A representative of such a class is the induction of the enzymes Gal-1-P uridyl transferase by galactose in cells growing on glycerol which Koyoshi Kurahashi (Science, Vol. 125, pp. 114-116, 1957).

The metabolic pathway in this case is given by Fig. 1. We postulate that the repressor is UDPGal, and that both galactose and UDPGal react reversibly as follows



This is an exceptional system inasmuch as the repressor UDPGal is made not only from the precursor, galactose, but is also made in cells which are grown on a carbon source other than galactose (in this case glyceral) from UDP glucose. Therefore, this is understandable that by raising the concentration of galactose we may not appreciable increase in the concentration of UDPGal, even in the ...

According to our theory, galactose is a real inducer of this enzyme because the equilibrium constant, K_{gal}, has a low value. The next enzyme along this pathway, Gal-1-puridyl transferase is, according to the terminology of this paper, a coupling enzyme, E_o, because it couples the metabolite galactose to the carrier, UDP.

Even though one must not generalize - even from one case which is more or less established - one is tempted to suspect that in general the carrier is a nucleotide. One would then assume that the metabolite combines with the head of the enzyme of which it is a substrate, and the nucleotide combines with the head of the template.

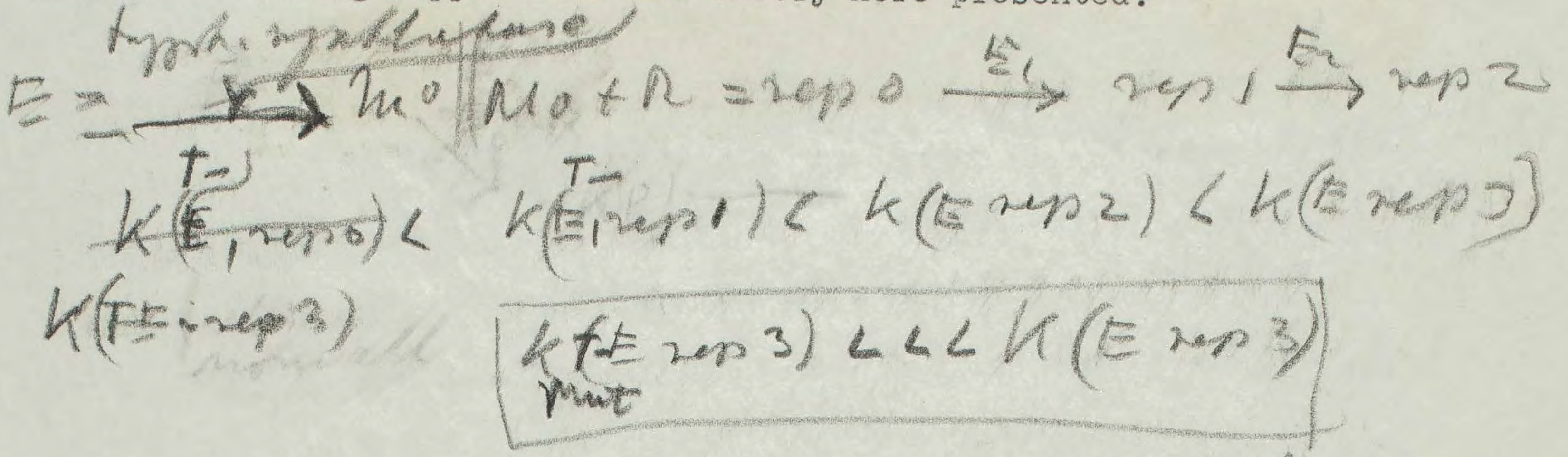
Constitutive strains and Biochemically deficient mutants

It seems clear that, from the point of view of the theory here presented, the classification of enzymes into inducible and non-inducible is not an adequate classification since all enzymes might be inducible by the precursor of their product if we define this precursor to be coupled to the carrier resulting in the appearance of a repressor.

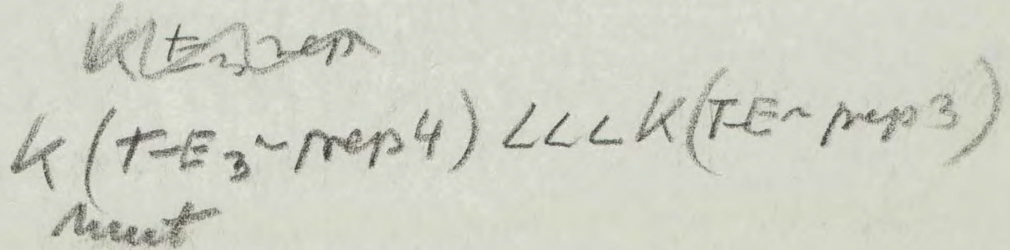
The wild type of coli in which the enzyme, β-galactosides can be induced by exposure to lactose or thiomethyl galactoside possesses a very low level of this enzyme when it is grown in minimal medium in the absence of an inducer. This strain can mutate and the mutant strain has a very high level of the enzyme, even in the absence of an inducer. Such strains are customarily called constitutive strains. The most likely interpretation of this phenomenon is as follows: the enzyme, β-galactosidase, is strongly repressed in the wild type because it makes an inhibitor, rep(m), in which the equilibrium constant K rep(n) with respect to

combining with the template-enzyme-complex is very small. If a mutation occurs and if the mutant in this binding constant is change from a low value in the wild type to a high value in the mutant, then the enzyme, β-galactosidase, can be expected to be produced at a high rate.

A similar interpretation must be given, I believe, to a most interesting phenomenon reported by Yanowsky and his co-workers which furnishes strong support for the theory here presented.



Suppressor a mutation in T_3

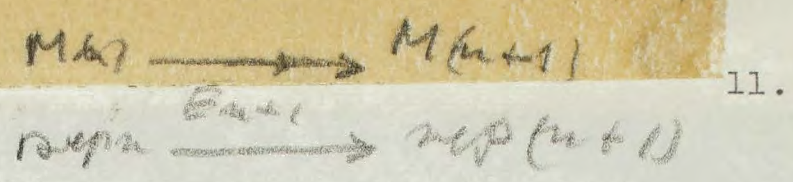


Yanowski

What is CRM

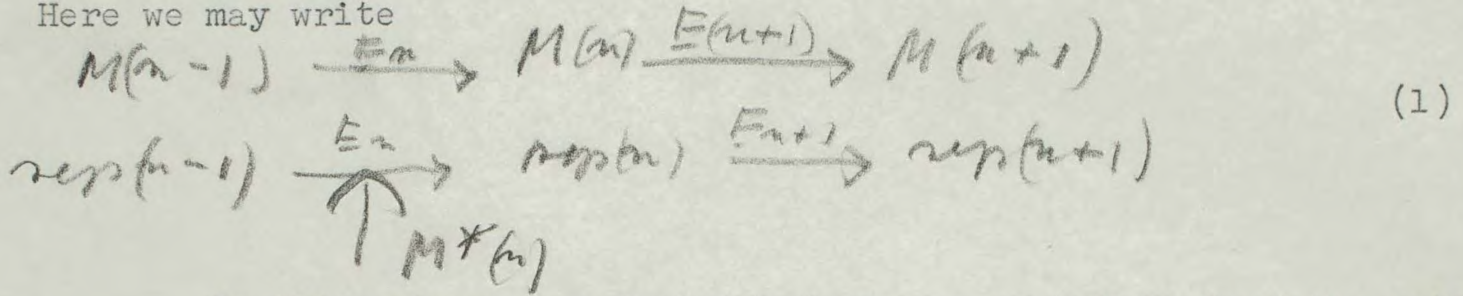
Typical cases of enzyme induction which are of greater interest to us ~~are found~~ not among chemical analogues of the precursors of the metabolite, M - which are in most cases also precursors of the vector, rep, but rather among the chemical analogues of the postcursors of the metabolite, M; i.e. the degradation products and ~~their~~ derivatives of the metabolites.

~~In the metabolic pathway which starts out with M and rep-O, we may assume that the enzyme-template-complex, T(n), combines reversibly~~



11.

Let us now consider M(n), a postcursor of M - n steps removed. Here we may write



(1)

In this scheme, as stated before, we postulate that the cell is not able to make a repressor out of the metabolite, M(n). This is the one salient fact that leads to the expectation that the rise of concentration of the metabolite, M(n) - or exposing the bacteria to a chemical analogue, M*(n) of M(n) - will enhance the rate of production of E(n+1).

PARAGRAPH

This may be seen as follows: The production rate of E(n+1) is repressed by the repressors, N(1) and N(+1), and possibly also although to a lesser degree by rep and rep(-1), which is one further step removed.

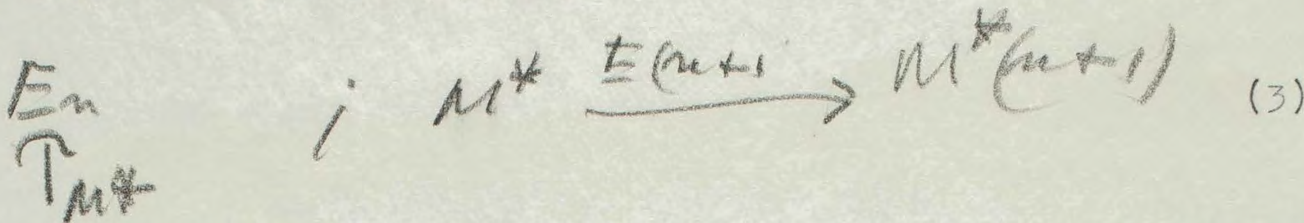
Either M(n) or its chemical analogue, M*(n) can be presumed to inhibit E(n), and thereby to lower the level of the repressors, rep(n) and rep(n+1). At the same time it can be expected to raise the level of rep(n+1). The sum total of these effects is likely to be an increase in f and the rate of enzyme production. The change in the levels of M(n-1), M(n) and M(n+1) may have an effect in the same or the opposite direction, depending on whether we expose the bacteria to M(n) or to M*(n), but at least if the constants for the enzyme-template-complex, T-E(n+1), are high enough, so that for this template-enzyme-complex we have

$$\frac{M(n)}{K(n)} \ll 1 \quad \text{and} \quad \frac{M(n+1)}{K(n+1)} \ll 1 \qquad (2)$$

then the effect of these ... changes can be neglected.

The upshot thus is an increase in the enzyme, E(n+1).

If M*(n) is a close enough chemical analogue of M(n) to be handled by the enzyme E(n+1), which it induces by its presence, this enzyme might catalyze the reaction



(3)

I presume this to be the explanation for the remarkable fact that bacterium like P-fluorescen may be able to oxidize fifty different ~~products~~ compounds and that its degradation products can induce some 200 enzymes.

[illegible handwritten annotation: "... adaptation give examples from Stanier ... 200 enzymes ..."]

INSERT

Drug tolerance

10^6 genes

Antibody Story -- Godd theory

The enzyme E(n) is not;far from being saturated by M(n). The metabolite M(n) is maintained at a certain level which depends on the number of intact templates that produce E(n). If all of the templates are intact, the stationary level of M(n) is determined by the rate at which the enzyme E(n) converts M(n) to M(n+1). If a certain fraction of the templates which can produce the enzyme are impaired so that the enzyme level is 10-20% low, then the level at which M(n) is maintained is higher, and we shall assume that it will now be determined by the rate at which the kidneys excrete M(n) or a derivative of it. Thus, when an antigen is injected for the first time, the following will happen:

The antigen will combine with all of the enzyme E(n) which is present and the level of M(n) will rise at a rate which is determined by its production and by its elimination through the kidneys. What about effect of increase M(n) level on rate of enzyme production? Does this not necessitate assumption of the destruction of a large fraction of the templates? Or can we explain the shortened latent period after the secnnd injection by the fact that there are a number of templates now ready to produce antibody -- this seems to be the preferable assumption. Accordingly, the picture now shapes up as follows:

When the antigen is injected, the hapten H* attaches itself to the enzyme E(n) and the enzyme will be destroyed. In this way all of the enzyme present is removed at the time when the antigen is injected. As a result of this now M(n) will rise at a rate which is determined by the rate of its production by the preceding enzyme, E(n-1) and by the rate at which it is eliminated through the kidneys. Fresh enzyme will be produced   No free enzyme will be present in the cell as long as antigen is present.

Similarly the production of Rep(n) may be halted and the concentration of Rep(n) will also rise in the same way as the concentration of M(n). The balance of these two processes will determine the rate at which the enzyme will be produced by the enzyme-template-complexes which are free. As long as antigen is present, however, it will tie up all enzyme-template-complexes within the cell, and no enzyme will be produced until antibody appears, and then the antigen is eliminated in a fairly short time by the reticulo-endothelial system. It is a basic assumption of this theory that when the antigen combines with the enzyme-template-complex, to which it may remain fixed for a matter of days, After the antigen dissociates off it will leave behind a certain fraction of the templates impaired in such a manner that these templates - whether still producing specific protein - the antibody which can combine with M(n)* and the antigen containing the hapten, M(n)* no longer can form the enzyme. The equilibrium constant of the template, both for Rep(n) and for M(n) because the fit is no longer perfect, we assume is increased for the damaged templates. Where before the damage occurred, we had K(rep) $\gg$ K(M(n)) compared to K(M(n)), now the two might be somewhat more comparable.

$$K(rep) \geqq K(M(n))$$

Thus, within a few days, as more and more antigen dissociates off the templates (?), antibody will be formed and in a number of days antibody titre is sufficient to eliminate the antigen.

When the antigen is eliminated, enzyme begins to form again and the titre of Rep(n) and of M(n) return to normal. The antibody titre reached after the first injection depends on the fraction of the templates that have been impaired and the value of the equilibrium constants of the impaired template.

When antigen is injected for a second time and ties up the enzyme, as soon as the level of M(n) reaches a certain value, the impaired templates (which will no longer combine with the hapten, M(n)* or which are no longer able to tightly hold the antigen-containing hapten, M(n)*, will begin to make antibody at a high rate, corresponding to the high level of M(n). This, however, will not last long for M(n) is an inducer of the enzyme and so the enzyme level will fairly rapidly rise and, even assuming a few per cent destruction of templates as a result of the second injection, the enzyme level will come back to normal and antibody production will fall to close the rate at which it was before the first injection.

If one starts out in thought with a bacterial strain that at some early point of evolution stocked with the synthesis of the basic metabolite, M, of the repressor, rep 0, but did not possess any enzymes, E(n), that could produce postcursors of M and postcursors of rep 0, and if one then considers what might have happened if in certain circumstances mutations occur in the template of E or E-0 which change the value of the constants, K(E) and K(E-0), then it is possible to see how the enzymes, E-N, arose as a result of suppressor mutations under selection pressure. This subject will be dealt with in another paper. For our purposes here, it is enough to say that, once such a system for stabilizing the rate of enzyme ~~systex~~ synthesis has somehow arisen during evolution, it will persist in a strain of bacteria even if the strain is grown over a long period of time in a mimimal medium because of the selection that would operate against a mutant which loses one of these enzymes E(n). Imagine, for instance, a mutant which loses E(1). Such a mutant would produce too much of the enzyme E_0 that produces rep 0, and therefore the production rate of E_0 would be repressed to the point where the mutant would be selected against in minimal medium. In the system here contemplated there is only one coupling enzyme, E_0, within an unbranched biosynthetic pathway of the sort described. This makes an important difference for the response of the bacteria to a metabolite which is a precursor of the basic metabolite, M, and which therefore might be rapidly converted into the repressor, rep 0. In

Last chapter