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Induced Enzyme Synthesis

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I. INTRODUCTION

A USEFUL hypothesis of modern genetics and that forming the basis of the preceding papers is that the genes (specifically, the cistrons) within a cell determine which proteins among all possible proteins a given cell can conceivably synthesize. Such genes are thus considered as the primary determinants of a given cell's allowance of proteins.

There are, however, other determinants of this allowance which, although secondary to genetic control, are of interest in any consideration of the mechanism of protein synthesis in that they specifically affect the synthesis of a single protein. Three phenomena have been sufficiently delineated to exemplify such secondary specific determinants. These are:

(1) Specific antibody synthesis resulting from exposure of cells to a given antigen.
(2) Induced enzyme synthesis.
(3) Repression of enzyme synthesis.

This paper is concerned solely with the last two phenomena, which quite possibly represent two forms of the same basic event.

Induced enzyme synthesis can formally be defined as the increase in the ratio of the rate of synthesis of a given enzyme to the rate of synthesis of total cell protein resulting from exposure of cells to compounds (inducers) which are identical or structurally related to the substrates of the given enzyme.[1] The vast majority of known instances of induced enzyme synthesis is derived from microorganisms, particularly the bacteria. This disproportionate representation is probably not real, for we have some reason to suspect that enzyme induction is operative in most cell types. Rather, it would seem that our observations are selected by the experimental techniques available for the manipulation of bacterial and fungal cell populations which have not, until very recently, been available for the culture of other cell types. A second consequence of this precision of bacterial manipulation is that the best-characterized and interpretable induced enzyme systems are found in the bacteria.

Induced enzyme synthesis in bacterial populations has been known for approximately seventy years, although, during a majority of its observed lifetime, this phenomenon has been shrouded in a teleological disguise by being named "enzymatic adaptation." It is only in the past decade that quantitative studies have allowed one to define this phenomenon as an induced enzyme synthesis not necessarily related to any increase in fitness of the cell in which it occurs.

Even in bacterial populations in which there are the most numerous examples of enzyme induction, it is quite clear that the majority of known enzyme-forming systems cannot be classified as inducible in that the enzymes are formed at considerable rates in the absence of exogenous inducers, and this rate of synthesis cannot be specifically increased by exposure of cells to their substrates or such structural analogs of these substrates as have been tested. Such enzymes are often referred to as constitutive enzymes in order to differentiate them from the induced variety. It must be stressed that the terms induced and constitutive do not describe the properties of an enzyme *per se*, but rather describe the properties of an enzyme-forming system. Thus, the same protein molecule can result from an induced enzyme synthesis in one bacterial population while being the resultant of constitutive enzyme synthesis in another, genetically distinct, bacterial population.

There is then an apparent dichotomy among enzyme-forming systems, being either inducible or constitutive. While at present the data are not sufficient for a unique explanation of this dichotomy, an attempt is made here to correlate these two systems to a common working hypothesis, thereby making the questions to be asked more specific and, it is hoped, experimentally answerable. It would not be profitable for the purpose of this paper to review the many known systems of enzyme induction or, in fact, even one of these systems in all of its detail. (For this purpose, see references 2–11). Rather, emphasis is placed on the salient features of one system that are relevant to a precise experimental illustration of the definition of induced enzyme synthesis given above and to the apparent dichotomy between induced and constitutive synthesis. The system of choice for this purpose is the β-galactosidase of *E. coli*, since it has been analyzed in perhaps the most detail as regards the enzyme protein itself, its induced synthesis, and the genetic and physiological relationship between its induced and constitutive synthesis.

II. β-GALACTOSIDASE INDUCTION

(A) Characteristics of the Enzyme Protein[8]

Since studies of the induction process depend upon the measurement of enzyme activity as a measure of the amount of enzyme protein present at any given time, it is obviously essential that a direct correlation between these two quantities can be made for the variety of conditions employed. This, in turn, demands that the catalytic and structural parameters of the enzyme in question be sufficiently determined. The β-

galactosidase of *E. coli* has been subjected to such determinations and it is useful in the discussion of its induced synthesis if some of its basic properties are briefly described.

(a) The characteristic reaction catalyzed by β-galactosidase is represented in Fig. 1, namely, the hydrolysis of β-D-galactosides. Many glycosides have been tested as substrates or competitive inhibitors of this enzyme yielding the conclusion that the minimum requirement for affinity for the active site on the enzyme is the existence of the β-D-galactopyranosidic ring.[8] Whereas all of the β-D-galactosides tested are substrates, an interesting class of compounds, the β-D-thiogalactosides in which sulfur replaces the oxygen atom of the galactosidic linkage, function only as competitive inhibitors. The interest in these compounds lies in their capacity to function as inducers of β-galactosidase synthesis, without at the same time being hydrolyzed by the enzyme whose synthesis they induce.

(b) Purified preparations of β-galactosidase have been obtained which contain at most 1 to 2% contaminating protein.[8] This allows a direct correlation of the unit of catalytic activity* with the mass of enzyme, yielding the value of 1 catalytic unit per 3.0×10^{-9} g of protein. Since Cohn[8] has determined by equilibrium-dialysis methods that there is one active site per molecular weight 1.3×10^{5}, activity measurements can yield a determinination of the number of active sites. Thus, one catalytic unit equals 1.4×10^{10} such sites.

(B) Induction Phenomenon

A culture of *E. coli* growing in a medium of inorganic salts with a nongalactosidic carbon source, such as succinic acid, produces only trace amounts of β-galactosidase. The addition of a suitable galactoside to such a growing culture is immediately followed by a sharp increase of over 1000-fold in the rate of synthesis of this enzyme. This high rate of synthesis is maintained as long as the bacteria grow in the presence of the inducing galactoside (inducer). However, if the inducer is removed, the rate of synthesis falls directly to the original small value. The quantitative aspects of this situation are presented in Fig. 2. As shown in the left-hand graph of this figure, from 0 to 120 min the bacteria are growing in the absence of the inducer and the amount of enzyme per unit weight of bacteria is quite low, approximately 7 units per mg dry weight of bacteria, which is equivalent to 20 active sites per bacterium. However, immediately upon adding the inducer, one sees that the amount of enzyme in the culture increases rapidly against a background of constant growth rate. Thus, while the bacteria have only doubled in amount, the β-galactosidase activity has increased by a factor of approximately 1200. Plotted in this way, one observes the transition of the culture from one steady-state condition in which the inducer is absent (noninduced state) to a second steady-state condition with inducer present (fully induced state). The bacteria in the fully induced state contain 8.5×10^{3} catalytic units per mg dry weight of bacteria or approximately 24×10^{3} active sites per bacterium. This increase of about 1200-fold in the amount of enzyme per bacterium in the fully induced state over that in the noninduced state means that the rate of synthesis of β-galactosidase relative to the rate of synthesis of bacterial mass (i.e., the differential rate of β-galactosidase synthesis[12]) has increased via the induction process by the same factor of 1200.

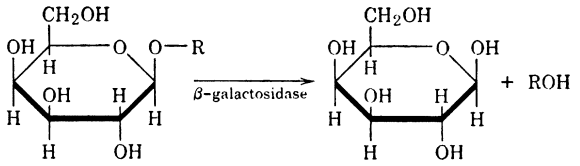


FIG. 1. Hydrolysis of a β-D-galactoside catalyzed by β-galactosidase.

It is this differential rate of enzyme synthesis which is of particular interest, since it is a more direct measure of the specific effect of the inducer on the rate of enzyme synthesis. One, therefore, wants to ask the question: how does this differential rate of β-galactosidase synthesis change during the induction process? This question is best answered by plotting the β-galactosidase activity of the culture *vs* the bacterial mass, a procedure which yields the right-hand graph of Fig. 2. Here, it is seen that the differential rate of β-galactosidase synthesis (i.e., the slope of the curve, P) changes from that of the noninduced state (7 units per mg dry weight) to that of the induced state (8.5×10^{3} units per mg dry weight) almost immediately after the introduction of the inducer, the transition taking place in less than 2 min, which is the minimum time detectable by the experimental techniques employed.

This high differential rate of synthesis remains constant as long as the inducer is present in the medium. However, as is shown in both graphs of Fig. 2, the inducer effect is readily reversible, since removal of the inducer immediately restores the differential rate of synthesis characteristic of the noninduced state, again with no appreciable change in the growth rate. Thus, in the case of β-galactosidase induction, it would appear that one has a system in which the synthesis of a given protein can be initiated or stopped by the simple addition or removal of a compound which, because it cannot function as a carbon or energy source, apparently does not influence the rate of synthesis of the vast majority of other cell constituents.

(C) Enzyme Induction as *de novo* Synthesis

Consider what is meant by synthesis as used in the preceding paragraphs. While it is true that the physical

* One unit of catalytic activity is defined as that amount of enzyme which will cause the hydrolysis of *o*-nitrophenyl-β-D-galactoside to occur at the rate of 1 mμmole/min at 28°C and *p*H 7.1 in $1.0\times10^{-1}M$ sodium phosphate and $2.7\times10^{-3}M$ *o*-nitrophenyl-β-D-galactoside.

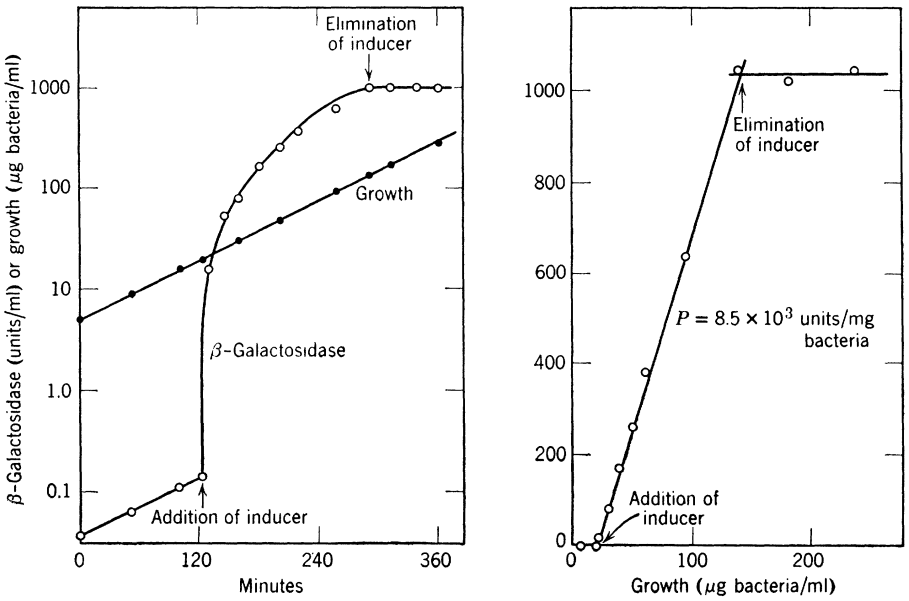


FIG. 2. Kinetics of β-galactosidase induction in *E. coli* ML 3. Inducer is isopropyl-β-D-thiogalactoside at 1.0×10^{-3} *M*.

and catalytic parameters of the active enzyme are sufficiently well known that one can calculate the mass of the active enzyme from the measurements of the catalytic activity present in the culture, it does not necessarily follow from this knowledge alone that an increase in such activity (and, therefore, of the mass of active enzyme) results from the *de novo* synthesis of the active enzyme. Within the context of the information so far presented, it is conceivable that the increase in active enzyme involved in the induction process results from the formation of a new protein structure (active enzyme) derived from inactive proteins present in the noninduced state. This transformation from inactive to active material could result (a) from a zymogen- to enzyme-type reaction typical of protease activations, (b) from the formation of a complex active protein made up of inactive subunits, or (c) from the addition of a few amino acids in peptide linkage to an inactive protein. In all of these cases, the induction process would have to be considered as independent of *de novo* synthesis in the sense that protein precursors of active β-galactosidase would be present in the noninduced state, and, consequently, that the total protein potentially or actually available as active enzyme would not necessarily be changed as a result of induction. It is, therefore, critical that one determine whether the induction phenomenon corresponds to the activation of protein precursors or to *de novo* synthesis, before it is possible to assess the value of this phenomenon as a tool for the study of general mechanisms of protein synthesis.

Although, by 1954, there were several lines of evidence which led, rather indirectly, to the tentative conclusion that β-galactosidase induction did indeed correspond to the *de novo* synthesis of this enzyme,[12] it was not until that year that conclusive evidence was available for the proof of this correspondence.[13,14] The direct proof of this correspondence came from experiments carried out in collaboration with Cohn and Monod[13], and simultaneously from the work of Rotman and Speigelman.[14]

The experiments consist of determining whether or not proteins present in noninduced cells are ever incorporated in or associated with the induced β-galactosidase protein by simply labeling the proteins in the noninduced cells with a suitable radioactive isotope and measuring the amount of radioactivity present in the enzyme when induced in nonradioactive media. Thus, in our experiments, *E. coli* were first grown in a simple salts medium in which the sole source of sulfur was S^{35} labeled sulfate. The amount of sulfate was so adjusted relative to the other components of the media that the bacteria stopped growing for lack of sulfate. In this starved condition, all of the radioactivity associated with the cells is contained within the trichloracetic-acid precipitable protein, and since no inducer was added to the medium, we have the condition of a unique radioactive labeling of the proteins of noninduced cells. This constitutes Step I (Table I) of the experiment.

In Step II, nonradioactive sulfate is added to the medium simultaneously with the inducer, methyl-β-D-thiogalactoside. This allows growth and induction to occur simultaneously. When the mass of the cells had increased so that 16% (aliquot *A*), 28% (aliquot *B*), and 43% (aliquot *C*) of the total growth had occurred in the nonradioactive inducing medium, aliquots of the bacterial population were removed, extracted, and, after purification of the β-galactosidase in these extracts, the amount of radioactivity associated with the enzyme determined.

For this experiment to succeed, it was necessary to develop purification techniques for the isolation of the

enzyme from less than one gram of cells of which only 0.16% was β-galactosidase. That this was possible is demonstrated by the data in Table I relevant to the isolation control in which unlabeled enzyme was mixed with a labeled extract of noninduced cells, such that the ratio of radioactivity to enzyme activity (K) in the mixed extract was the same as that for the crude extract of aliquot A. By comparing the K-ratio of the enzyme isolated from this reconstruction mixture with that isolated from bacteria grown and induced in the S^{35} labeled medium (fully labeled enzyme control), it is clear that the purification procedure yields an enzyme preparation which contains less than one percent bacterial protein contamination (0.4% in this case).

The enzyme purified from aliquots A, B, and C contained, respectively, 0.1, 0.8, and 0.1% of the radioactivity associated with the fully labeled enzyme. Since this is within the limits of the purification procedure as indicated by the isolation control data, and since these values do not progressively decrease with increase in the fraction of total growth occurring in Step II, it was concluded that less than 0.8% of the sulfur of the enzyme formed in Step II was derived from proteins present in the noninduced state (Step I). This result, taken in conjunction with the fact that, in aliquot A, the enzyme level was only 5% of that found in fully induced bacteria, indicates that if any protein precursor of β-galactosidase exists in the noninduced bacteria, its level must be less than 0.04% of that for β-galactosidase in fully induced bacteria or less than 10 precursor molecules of molecular weight 1.3×10^5 (i.e., the molecular weight per active site of β-galactosidase) per bacterium, assuming of course that such precursors contain the same percent sulfur as does β-galactosidase. This conclusion is enforced by the fact that Rotman and Spiegelman[14] found essentially the same results using C^{14} as the labeling material.

The results of these experiments allow one to conclude that, if there are any protein precursors existent in noninduced cells, they cannot form an appreciable contribution to the increase in active enzyme found upon the addition of the inducer, since it would take only a few seconds to form 10 new active sites per bacterium under the conditions of induction indicated in Fig. 2. Thus, when cells are exposed to inducer molecules the over-all rate of conversion of amino acids into a specific protein, β-galactosidase, is drastically and immediately increased, and consequently induced enzyme formation is equivalent to induced *de novo* synthesis. It would appear then, that the induction phenomenon should be accounted for in any working hypothesis that attempts an explanation of the general mechanism of protein synthesis.

TABLE I. Incorporation of sulfur into β-galactosidase synthesized by labeled *E. coli* cells in nonradioactive medium.

Experiment	Percent maximal enzyme level	$K = \frac{\text{radioactivity}}{\text{enzyme activity}}$	Percent of K for fully labeled enzyme[a]
Step I	0.06	(0.45)[b]	(100)
Step II—*A*	4.8	0.0050	0.1
B	32	0.0043	0.8
C	58	0.00072	0.1
Controls			
Fully labeled enzyme	100	0.45	100
Isolation control	(4.8)[c]	0.0018	0.4

[a] Corrected for basal activity.
[b] ( ) Basal level assumed to be equal to fully labeled enzyme.
[c] Reconstruction of Extract *A* by mixing unlabeled enzyme with a labeled extract of noninduced cells.

Two further conclusions resulted from our labeling experiments. The first stems from the preceding experiment and states that, in exponentially growing *E. coli* cells, the vast majority of non-β-galactosidase proteins are stable in the sense that they are not broken down to their constituent amino acids at rates that are appreciable in relation to the rates of synthesis. If the state of proteins within these cells consists of a continual synthesis from and breakdown to their constituent amino acids (i.e., state of "dynamic equilibrium"), then one would expect the β-galactosidase synthesized in Step II of the above experiment to be labeled with S^{35} as a result of the breakdown of the radioactive proteins. A simple calculation from the data of this experiment yields the conclusion that the average rate of breakdown of the non-β-galactosidase proteins must be less than one percent of their average rate of synthesis.

It is, therefore, not surprising to recall that, when the inducer is removed from an exponentially growing culture of *E. coli* cells (Fig. 2), the β-galactosidase formed while inducer was present remains constant, simply being diluted out in the exponentially increasing population. This would indicate that β-galactosidase is also stable once formed. Because of the possibility that this constancy of enzyme activity results from an equal rate of synthesis and degradation of β-galactosidase, and to test whether or not β-galactosidase is also stable in the presence of the inducer, we carried out further S^{35} labeling experiments.[13] These experiments yielded the expected conclusion that the β-galactosidase molecules in exponentially growing cells are stable in both the absence and the presence of inducers. Thus, it can be concluded that the induced *de novo* synthesis of β-galactosidase is, like the synthesis of other proteins in *E. coli*, essentially an irreversible process, and the so-called "dynamic state" is not a concept that need be involved in an explanation of the mechanism of such synthesis.

(D) Induction at the Cellular Level

While it is clear from the foregoing discussion that one may interpret the kinetics of formation of β-galactosidase activity during the induction of a bacterial population as the kinetics of *de novo* synthesis of this enzyme in that population, one can only extrapolate these kinetics to the cellular level if one makes the as-

sumption that all cells participate equally and simultaneously in this induced synthesis. Even though one can demonstrate a high degree of genetic homogeneity for the bacterial population being induced, there is no *a priori* promise that this assumption of equal and simultaneous participation of all cells is justified. It is, therefore, critical to the correct interpretation of the kinetics of induced enzyme synthesis in cultures that one have a knowledge of the distribution of rates of induced synthesis among the cells of the bacterial population.

There are two known types of conditions that can cause a heterogeneous response of a bacterial population when exposed to inducer molecules. The simplest of these types is a condition whereby the catalytic activity of the first enzyme molecules synthesized by a given cell increases the probability of synthesis of future enzyme molecules by that cell. As an example of this condition, consider a culture of *E. coli* whose growth has stopped because of exhaustion of glucose in the medium. If lactose (4-glucose-β-D-galactoside) is added to such a culture, it will have two functions: to induce the synthesis of β-galactosidase and, as a substrate of this enzyme, to provide the only available carbon and energy source necessary for cell synthesis. Under these circumstances, the induced synthesis of the first enzyme molecules must depend upon traces of enzyme already present or upon internal bacterial reserves. Since synthesis of the first enzyme molecules by any cell will increase the availability of carbon and energy for further synthesis by that cell, the cells which have a head start will increase their advantage. Thus, any initial heterogeneity in the population with regard to internal reserves or initial amount of β-galactosidase would be expected to result in an exaggerated heterogeneity in respect to enzyme content during the initial stages of growth on lactose. This expectation has been verified experimentally by Benzer.[15]

Elimination of this factor favoring heterogeneous response is quite simple and consists of using an inducer which is not a substrate of β-galactosidase (e.g., the β-D-thiogalactosides), or alternatively of employing mutant bacteria which cannot further metabolize the products of hydrolysis of the inducer, while at the same time providing a nongalactosidic carbon and energy source (e.g., succinic or lactic acid) for cell synthesis. These conditions have been defined as "conditions of gratuity" by Monod and Cohn[3] and are the conditions employed in the experiment illustrated in Fig. 2. Benzer[15] found that, in the induced synthesis of β-galactosidase under conditions of gratuity and at saturating levels of inducer concentration, the bacterial population exhibits a homogeneous reaction in that essentially all cells participate to the same degree. Hence, under these conditions, the kinetics of induction of a culture represent the kinetics of induction of single cells.

Although most readers of Benzer's work concluded that conditions of gratuity were a sufficient guarantee for the assumption of population homogeneity during induction, Benzer himself cautioned against applying this assumption to conditions of less than saturating concentrations of inducer even though the induction was "gratuitous." Indeed, a second more subtle factor causing heterogeneous response at less than saturating concentrations of inducer has been discovered by Rickenberg, Cohen, Buttin, and Monod[16] (also referred to in reference 9). Thus, these workers found that, in wild-type *E. coli*, the β-galactosidase induction can be divided into two separate processes. The first of these involves an active transport of the inducer into the cell such that inducer concentrations inside the cell are much greater than those in the medium. The second is the actual induction of β-galactosidase synthesis inside the cell at a rate determined by the internal concentration of inducer. The active transport of inducer is accomplished by a unit, called galactoside-permease, which has been demonstrated to have most of the characteristics usually associated with enzymes and whose synthesis is induced by many of the same compounds which induce β-galactosidase synthesis, again at a rate determined by the internal concentration of inducer.

This transport mechanism obviously introduces another factor that would be predicted to encourage a heterogeneous response of a population upon exposure to inducer, providing the probability that a given cell will produce its first permease molecule per smallest detectable time unit is small. Thus, those cells which do synthesize their first permease unit will have an increased internal concentration of inducer which will, in turn, increase the probability of synthesizing the second permease unit above that for the first. Thus, those cells which by chance are the first to synthesize permease units will increase their advantage and rapidly pass to the stage of saturating internal concentrations of inducer and maximum rate of synthesis of both the permease and β-galactosidase. The distribution of rate of enzyme synthesis in the bacterial population at any given time after addition of the inducer will thus depend upon the probability that a given cell will synthesize its first permease unit and upon the time interval between this event and when maximum rate of synthesis is reached.

Novick and Weiner[17] and Cohn[8] have analyzed the kinetics of β-galactosidase induction in the wild-type *E. coli*, which is inducible for both the galactoside-permease and β-galactosidase. They have found that these kinetics are consistent with the foregoing theory with the addition that the probability of synthesis of the first permease units is determined by the inducer concentration, and that the time interval between this event and when maximum synthetic rate is achieved is very small, even at the lowest inducer concentrations studied. This means that, at inducer concentrations well below saturation, the distribution of rates of enzyme synthesis at any given time is essentially an all-or-none distribution; i.e., cells are synthesizing enzyme either at the maximum rate or at the minimum noninduced rate.

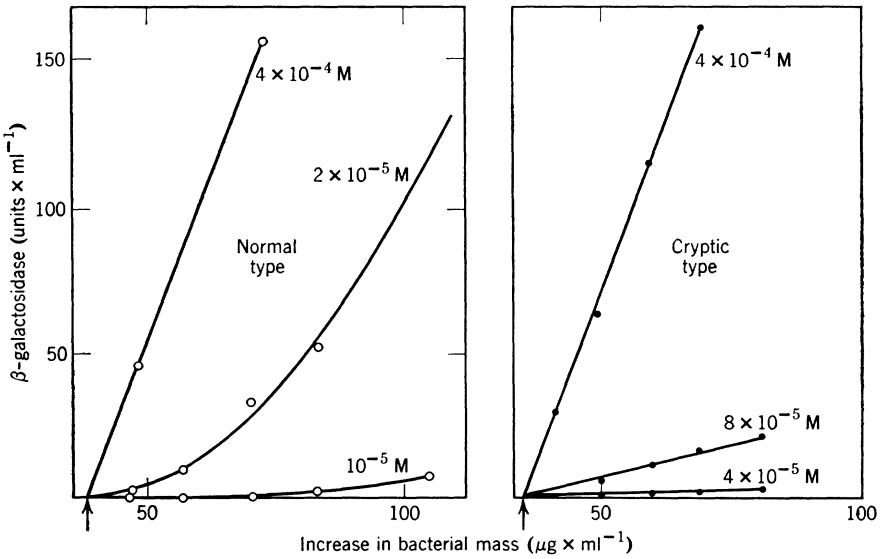


FIG. 3. Kinetics of β-galactosidase induction in normal *E. coli* and in the cryptic mutant. Inducer is isopropyl-β-D-thiogalactoside at the concentrations indicated above [from L. A. Herzenberg, "Studies on the induction of β-galactosidase in a cryptic strain of *Escherichia coli*," Biochim. et Biophys. Acta (to be published)].

As this induction at low inducer concentrations continues, a larger and larger fraction of the bacterial population is synthesizing enzyme at the maximum rate, and, consequently, a plot of enzyme activity *vs* increase in bacterial mass during the induction of such a culture will yield a curve with a slope (differential rate of synthesis) that increases with increase in growth to some final constant value (left-hand graph of Fig. 3). As the external inducer concentration is raised, the probability of synthesis of the first permease unit increases, and, consequently, the time duration of the phase of increase in differential rate of synthesis, or what may be termed the heterogeneous phase, decreases until it is no longer detectable. Under these conditions of saturating inducer concentration, for all intents and purposes, the distribution of rates of β-galactosidase synthesis in the population at any given time after addition of inducer is uniform, and, consequently, the kinetics of induction of the culture represents the kinetics of the individual cells.

However, it is quite clear that wild-type *E. coli*, in which both the galactoside-permease and the β-galactosidase are inducible, cannot be used to define the kinetics of induction of single cells at less than saturating concentrations of inducer. This could be accomplished if the galactoside-permease could be eliminated, thereby removing the last contributing factor toward heterogeneous response. The most convenient and complete method of such elimination is by the isolation and use of *E. coli* mutants which have lost the ability to form galactoside-permease, but retain the property of being inducible for β-galactosidase. Such mutants have been isolated and are commonly called inducible cryptics, in that, while β-galactosidase can be induced in these strains, because they lack the permease, the induced enzyme is essentially hidden from external substrate (e.g., lactose), the rate of hydrolysis in whole cells being limited by passive diffusion of the substrate.

One of these inducible cryptics (*E. coli* ML3) has been used extensively by Herzenberg[18] to define the kinetics of induction throughout the range of inducer concentrations that produce measurable increases in the rate of β-galactosidase synthesis. It is also the strain used in the experiment depicted in Fig. 2. An interesting result of the experiments of Herzenberg is that, for each inducer concentration tested, the differential rate of synthesis remains constant from the time of addition of the inducer, although the actual value of this rate is determined by the inducer concentration (Fig. 3). Under the assumption that, by employing conditions of gratuity and the inducible cryptic strains of *E. coli*, all factors encouraging a heterogeneous response during induction have been eliminated, one can interpret these kinetics of constant differential rate of synthesis for the culture as representing the kinetics of induction of individual cells. It must, however, be noted that, while this somewhat tortuous path of discovery and elimination of the sources of heterogeneous response during the induction process in *E. coli* wild-type makes this assumption quite reasonable, there is at present no direct experimental evidence available that completely justifies it.

If one admits this assumption, then the kinetics of constant differential rate of β-galactosidase synthesis during induction of the inducible cryptic strains (Fig. 3) allows one to conclude that the number of enzyme-forming units per cell that are activable by the inducer remains constant during induction. For, if the number of these units per cell should change during induction, then, in the presence of a constant subsaturating internal concentration of inducer, the differential rate of synthesis should change proportionately. The precision of the conclusion as to the constancy of β-galactosidase

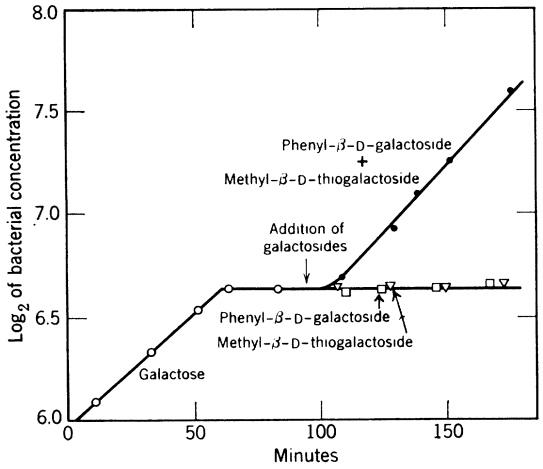


FIG. 4. Growth of *E. coli* ML 30 on galactose and the indicated galactosides demonstrating the independence of inducer and substrate functions.

forming units per cell is equivalent to the precision of the experimental observation of constant differential rate of synthesis. Consequently, if any change in the number of these units occurs during induction, it must occur in every cell of the population within two minutes of the moment the inducer is added. Since this seems extremely unlikely, it can be inferred that the same number of enzyme-forming units exist in noninduced cells as in induced cells, these units being active only in the presence of the inducer.

(E) Independence of Substrate and Inducer Functions

A corollary to this conclusion is that the catalytic activity of β-galactosidase is not functional in its own synthesis; i.e., the process of enzyme induction is independent of enzyme action. The kinetic derivation of this postulate is confirmed by the observation that the property of being a substrate for β-galactosidase is neither a sufficient nor necessary condition for a compound to function as an inducer. Thus, phenyl-β-D-galactoside is an excellent substrate of β-galactosidase, but is not functional as an inducer. Methyl-β-D-thiogalactoside, on the other hand, is not a substrate of the enzyme, but is an excellent inducer. The inverse relationship of these two compounds is demonstrated quite clearly by a simple growth experiment shown in Fig. 4. An inducible strain of *E. coli* was grown in a medium containing galactose as the sole carbon source until growth stopped (*ca* 60 min) as a result of depletion of galactose. Since galactose does not induce β-galactosidase synthesis, these starved cells contain only trace amounts of the enzyme. After waiting a short time to insure complete starvation, the starved cells were divided into three parts, and the galactosides indicated in Fig. 4 were added as the sole carbon source. The cells did not grow in the presence of methyl-β-D-thiogalactoside because, although it can induce β-galactosidase synthesis, it is not a substrate and, consequently, cannot function as the necessary carbon source. No growth is observed in the presence of phenyl-β-D-galactoside, because it is not an inducer of the enzyme necessary for it to function as a carbon source. However, when the two galactosides are added together, growth occurs since methyl-β-D-thiogalactoside functions as the inducer and phenyl-β-D-galactoside serves as the substrate, yielding galactose, the necessary carbon source.

In addition to the qualitative data as to independence of substrate and inducer functions, quantitative measurements of the ability of many thiogalactosides to induce β-galactosidase synthesis and the ability to complex with the active site of this enzyme show no correlation. Thus, phenyl ethyl-β-D-thiogalactoside is an extremely good competitive inhibitor of β-galactosidase action ($K_I = 1.5 \times 10^{-5} M$), but can induce the synthesis of β-galactosidase to a rate that is only one-eightieth that obtainable with methyl-β-D-thiogalactoside, although this latter compound is about 800-fold less efficient as a competitive inhibitor ($K_I = 1.2 \times 10^{-2} M$). On the other hand, melibiose, an α-galactoside which is neither a substrate nor an effective competitive inhibitor of β-galactosidase, is quite effective as an inducer of this enzyme. Thus, it would appear that, in activating the enzyme forming units within the cell, the inducer reacts with some material having a different specificity than that associated with the active site of β-galactosidase.

There remains, however, one minimum structural requirement common both to inducers and to substrates or competitive inhibitors—namely, that they contain the galactopyranosidic ring structure.† The inference, then, is that the site at which the inducer reacts to activate the enzyme-forming unit is structurally similar but not identical to the active site of β-galactosidase.

In concluding this experimental definition of induced enzyme synthesis, the main conclusions that have been obtained are listed below.

1. The induction process involves the complete *de novo* synthesis of the enzyme from its constituent amino acids.

2. Induced enzyme synthesis is a virtually irreversible process, the enzyme being stable in the presence or absence of inducer.

3. The number of enzyme-forming units per cell remains constant during the induction process; that is, the inducer does not change the rate of synthesis of enzyme-forming units, but simply activates such units.

4. The site at which the inducer reacts to activate the enzyme-forming unit is structually similar but not identical to the active site on the enzyme.

† This is strictly true only if the *C*6 carbon is not considered part of the ring, since the α-L-arabinosides (which are derivatives of β-D-galactosides lacking *C*6) exhibit the property of being weak substrates for β-galactosidase.

In addition to the above conclusions, one should mention another of equal importance but which, at present, cannot be derived from data on β-galactosidase induction. It is that the inducer acts as a catalyst in the sense that one molecule of inducer may cause the formation of more than one molecule of enzyme. This observation comes from experiments concerning the penicillin-induced synthesis of the enzyme penicillinase in *Bacillus cereus*.[11] It should be noted that each of the foregoing conclusions has been experimentally confirmed in only one, or, at the most, a very few systems and that, consequently, their generality remains to be shown.

III. CONSTITUTIVE SYNTHESIS OF β-GALACTOSIDASE

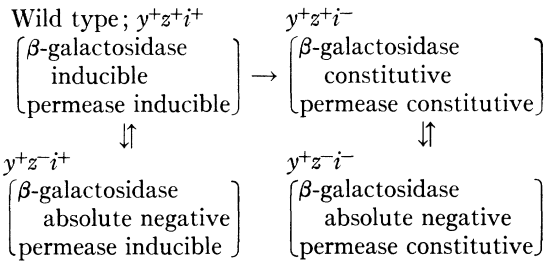
Before analyzing some of the hypotheses attempting to explain the mechanism of induction, it is useful to first describe the phenomenon of the constitutive synthesis of β-galactosidase in *E. coli* and the origin of the bacteria responsible for it.

If one examines a β-galactosidase constitutive strain of *E. coli* that is growing in the absence of an inducer, one will find that β-galactosidase synthesis occurs at a very high rate, 1000-fold or more greater than is found for the inducible strains growing under the same conditions. Furthermore, the addition of an inducer to such a constitutive culture does not augment this already high rate of synthesis. Thus, the constitutive strains synthesize β-galactosidase in the absence of inducer at rates which are apparently maximal for these cells and which are approximately the same as the maximum rates of induced β-galactosidase synthesis found in the inducible strains.

The question that is immediately raised in such a comparison of constitutive and induced β-galactosidase synthesis is whether one is concerned with the synthesis of the same protein molecule in each case or whether two different proteins are included in the above use of the term, β-galactosidase, one being synthesized in inducible strains and the other in constitutive strains. This question has been answered with some certainty in favor of identity.[3,19] Thus, the titration of catalytic units precipitable by a given quantity of specific anti-β-galactosidase sera yielded the same results with enzyme preparations derived from constitutive bacteria as with those derived from induced cells, whether the antibody was formed in response to the induced or to the constitutive enzyme. This not only demonstrates the antigenic identity of the two enzymes, but also indicates that their turnover numbers (catalytic units per molecule) are the same. Similarly, an extensive comparison of the kinetic constants for several substrates, for sodium- and potassium-ion activation, and for thermal inactivation of the β-galactosidase in these two preparations did not reveal any measurable difference.

Taking these observations in good grace as sufficient evidence for the identity of the β-galactosidase in constitutive and induced cells, one should expect the basic genetic unit or units necessary for the synthesis of this protein to be common to both cell types, assuming, of course, that one accept the tenets of the DNA-protein doctrine presented by Levinthal (p. 227). What then is the genetic difference between inducible and constitutive *E. coli*?

The following series of mutation sequences indicates the relationship between inducible and constitutive strains[16,20,21]:



In this scheme, the genetic units have the following meaning:

1. $i^+ \rightarrow i^-$ designates the mutation involving a change of state from inducible (i^+) to constitutive (i^-) for both permease and β-galactosidase.
2. $z^+ \rightarrow z^-$ designates the mutation involving damage to the z^+ unit necessary for β-galactosidase synthesis such that the enzyme cannot be made.
3. y^+ indicates the unit necessary for the synthesis of the galactoside-permease, a $y^+ \rightarrow y^-$ mutation (not shown) being that involved in formation of the cryptic, permease negative mutants previously mentioned.

This genotypic interpretation of the mutation data is consistent with data from recombination studies[22] and, as with a unique type of *cis-trans* test demonstrating that y^+ and z^+ involve different cistrons.[23]

From the fact that the β-galactosidase-proteins synthesized by constitutive and induced bacteria are identical, and from the genetic evidence demonstrating that the change resultant from mutation of inducibles (i^+) to constitutives (i^-) involves genetic units (cistrons) different from those involved in the formation of absolute negatives (z^-), one comes to the conclusion that the mechanism of synthesis of β-galactosidase in induced and constitutive cells does not differ in its essentials—that is, the same amino acids and the same mechanisms of ordering such amino acids are employed in each case. Though slightly more specific, this conclusion is equivalent to the general unitary hypothesis for enzyme synthesis emphasized by Cohn and Monod.[4]

Thus, in addition to conforming to the conclusions drawn directly from the induction phenomenon (Sec. II), any useful hypothesis for the induced enzyme synthesis must also explain the inducible to constitutive transition via the agency of a single-step mutation not involving a change in the basic genetic units determining the structure of the enzyme.

IV. HYPOTHESES FOR INDUCED ENZYME SYNTHESIS

The point of departure for all hypotheses purporting to explain enzyme induction within the context of the unitary principle is the choice between two possibilities: (1) an inducer is an essential part of the minimum requirements for rapid enzyme synthesis (i.e., the "generalized induction principle"[4]); or (2) the converse, inducers are *not* part of these minimum requirements.

Since there exists no direct experimental evidence which determines this choice, it is necessary to analyze the consequences of both possibilities. A number of schemes have been developed that invoke the inducer as an essential element of the minimum requirements for enzyme synthesis.[4–6] It is not proposed to analyze these here, since they either do not attempt to explain at what point and in what manner the inducers act in the over-all conversion of amino acids to enzyme molecules,‡ or they are not consistent with the conclusions formed in Sec. II from data on β-galactosidase induction.§ Rather, a model of enzyme synthesis is presented here in which the necessity of inducer participation can easily be visualized and which is consistent with the conclusions drawn thus far. The initial assumption in this construction is that the inducer is neither necessary nor functional in determining the amino-acid order of the induced enzyme, but instead acts to increase the rate of formation of its secondary or tertiary structure. This assumption has its basis in the coding theory of DNA function in which the amino-acid order in a given protein is uniquely determined by the nucleotide order in the DNA of some functional gene (cf. Levinthal, p. 249).

A diagrammatic representation of how an inducer might exert a catalytic function in tertiary structure formation is given in Fig. 5. In this diagram, the semicircle of capital letters represents a template of unspecified chemical composition (DNA, RNA, or other) whose structure is determined by a functional gene and which in turn determines the order of amino acids (lower-case letters) making up a specific polypeptide. Each capital letter then represents a code symbol for one amino acid, and it is assumed that the physical structure which determines a given code symbol specifically binds only one amino acid. Peptide-bond formation between amino acids is imagined to occur on this template with the condition that the binding force between a given amino acid and its code structure is not lost by the formation of the polypeptide. Consequently, polypeptide and template form a relatively stable complex, which momentarily may assume any one of many partially dissociated states. Certain of these transitory, partially dissociated configurations will be favorable for the formation of the tertiary structure necessary for enzyme formation (represented here by the formation of a disulfide bond via the oxidation of two sulfhydryl residues in the polypeptide chain). The formation of such tertiary structure is assumed to be concomitant with dissociation of polypeptide and template, thus allowing the formation of free enzyme.

With no inducer present, such favorable states are considered to be extremely short-lived, so that the probability of formation of the disulfide bond pictured in Fig. 5 is quite small. As a consequence, the rate of enzyme synthesis is very low.

The assumed function of the inducer, or some product derived from it, (X), is to stabilize the favorable configurations by interacting with them to form a complex of the type represented by the middle left-hand structure in Fig. 5. Such a stabilization would result in an increase in the probability of the critical disulfide-bond formation and, therefore, in the rate of enzyme formation.

This mechanism is offered more as an aid in visualizing how one can imagine the necessity of inducer participation in protein synthesis, rather than as a unique representation of this possibility. However, it is consistent with the conclusions developed concerning β-galactosidase induction. Thus, it accounts for the *de novo* synthesis of β-galactosidase during induction, since

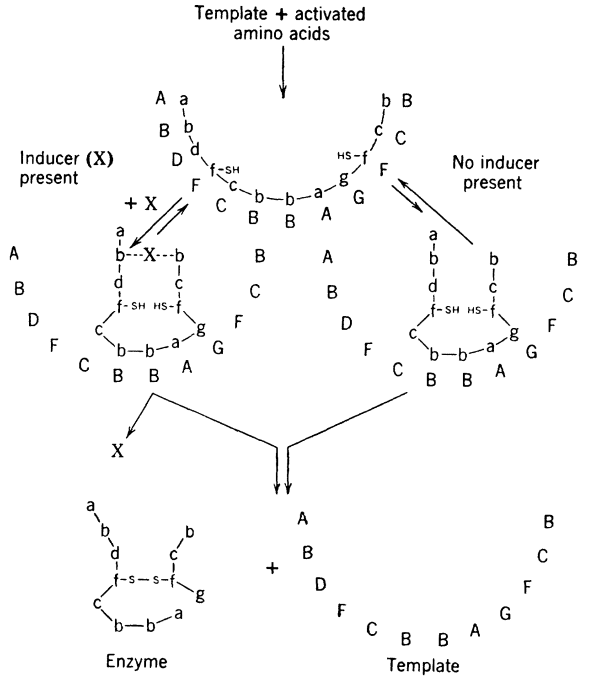


FIG. 5. Model for inducer function under the generalized induction hypothesis.

‡ The "organizer" hypotheses[4,5] insist that a derivative of the inducer (the "organizer") is an essential in enzyme synthesis but leave quite unspecified the mechanism by which such a derivative catalyzes enzyme synthesis.

§ The mass-action hypothesis by Yudkin[24] and its extensions[6] demand that enzyme induction be a *reversible* process in which the specificity of inducers of enzyme synthesis parallel the specificity of substrates and competitive inhibitors of this enzyme, conditions which are not consistent with the data derived from β-galactosidase induction (Sec. II).

the level of polypeptide precursors of the enzyme in noninduced cells could be no greater than the number of templates per cell. Indeed, the calculated limit of ten or less such precursor molecules per noninduced cell is not an unreasonable limit for the number of templates per enzyme per cell. The model also allows for an essentially irreversible synthesis of β-galactosidase during the induction of growing populations; for the activation of enzyme-forming sites (templates) by inducers and the constancy of such sites during induction; and, finally, for the similarity but nonidentity of the specificity of inducers and substrates (or competitive inhibitors) of the enzyme. It is perhaps of interest to note that, insofar as reversal of the synthetic reaction in the model is limited by competition of activated amino acids and enzyme for the template (the enzyme having a very small affinity for the template as compared to the precursor polypeptide), the rate of any possible reverse reaction would be predicted to be greater under conditions of low levels of activated amino acids (e.g., in nitrogen-starved cells) than when these compounds are plentiful (e.g., in fast-growing cells.)

With respect to this model, or any model that invokes an inducer as an essential in the minimum requirements for the synthesis of a given enzyme, the two genetic states of inducible and constitutive β-galactosidase synthesis can be interpreted by assuming that constitutive cells contain an endogenous inducer, and that inducible cells are unable to synthesize such an inducer for lack of a necessary enzyme. On this basis, the mutation of an inducible strain to a constitutive can be viewed as a repair of a damaged genetic unit responsible for the synthesis of the postulated enzyme.

Turning now to the second possibility—namely, that an inducer is not a necessary component of the minimum system for enzyme synthesis—one must accept the constitutive condition as representing this minimum system. A model built for such a system could be like that given in Fig. 5, with two important differences: (1) The favorable state leading to tertiary structure could be stabilized by the form of the template itself, thus eliminating the need for inducer; and (2) the affinity of the activated amino acid for its code structure on the template would have to be lost as a result of peptide-bond formation with its nearest neighbors. The latter condition could be allowed for if one supposes that the activated amino acid consists of the amino acid covalently linked to a residue, *R*, which is unique for each amino acid, and that the code structure on the template has an affinity for *R* rather than the amino acid itself. If peptide-bond formation entails the breaking of the *R*-amino acid bond, then the polypeptide thus formed is quite free to separate from the template.

However, regardless of the model one builds for the synthesis of an enzyme without inducer (endogenous or exogenous) participation, the induced enzyme synthesis must differ from constitutive synthesis, either by a difference in type of template or by the existence of an inhibitor of normal synthesis (i.e., constitutive type synthesis) in the inducible strain, an inhibition which is relieved by the addition of an exogenous inducer. In the first of these possibilities, one must imagine that mutation from inducible to constitutive invokes a change in template type from one requiring an inducer for synthesis (i.e., as in Fig. 5) to one in which no inducer is required. Furthermore, since the β-galactosidase molecules synthesized constitutively and by induction are identical, one must suppose that this change in template type does not involve a change in the order of the amino-acid code. This seems to be asking a lot from a single mutation, but since almost nothing is known of the template structure, one cannot say, *a priori*, that such a change is impossible.

The second possibility—namely, that induction is the release of inhibition of enzyme synthesis—is actually quite an old idea, but in the past it was generally offered facetiously as the *bête noire* of generalized induction theories, since it could not be eliminated. However, recent developments demand more positive consideration of this idea. The source of these developments lies in the observation of what Vogel[25,26] has termed "enzyme repression." Repression is the inhibition of the differential rate of synthesis of an enzyme resulting from exposure of cells to a given substance ("repressor"). The word repression was adopted simply to avoid confusion with the term "enzyme inhibition," which by long usage implies the inhibition of the catalytic function of a given enzyme and not of its synthesis. One of the best examples of enzyme repression is observed among the constitutive enzymes included in the biosynthetic pathway leading to the synthesis of ornithine, citrulline, and finally of arginine in *E. coli*. Thus, Vogel[25] found that arginine is a specific and very effective repressor of the synthesis of acetylornithinase. Similarly, Gorini and Maas[27] have shown that arginine also represses the synthesis of ornithine transcarbamylase. Perhaps the most significant implication of these observations is that there exists within the cell a mechanism of feedback control between the result of the catalytic action of the group of enzymes in a biosynthetic pathway (in this case, the synthesis of arginine) and the synthesis of these enzymes. For present purposes, however, they give plausibility to the supposition that the inducible strains could represent a case of repression of the constitutive synthesis of β-galactosidase by endogenous repressors present in such strains, but not in the constitutive strains. Indeed, it is known that the constitutive synthesis of β-galactosidase in *E. coli* can be inhibited by exposure of cells to galactose or one of a variety of β-D-galactosides[4]; i.e., in the constitutive strains, these substances act as exogenous repressors of enzyme synthesis.

One may, therefore, term this second possibility the repressor hypothesis. It states that in constitutive strains enzyme synthesis occurs without the aid of any inducer; that such synthesis is inhibited in the inducible

cells because of repressor substances synthesized by these cells and not by constitutive cells; and that an inducer destroys this inhibition of enzyme synthesis either by combining with the repressor to yield a non-inhibiting complex, by competing with the repressor for some site on the template (or template-polypeptide complex)—which, if occupied by the inducer, does not inactivate the template, but if occupied by a repressor does so inactivate—or by inhibiting the synthesis of unstable repressors.

The three alternative explanations of induced and constitutive enzyme synthesis can be summarized as follows:

1. Generalized-induction hypothesis. Inducers are necessary components for enzyme synthesis both in constitutive and in inducible cells. Endogenous inducers are synthesized by constitutive cells but not by inducible cells, which must, therefore, receive exogenous inducers for enzyme synthesis.

2. Different-template hypothesis. Two different types of template exist for enzyme synthesis. In constitutive cells, the templates function without the aid of inducers. In inducible cells, the inducers are necessary to activate the template (as in Fig. 5). No endogenous inducer or repressor is assumed. It is assumed that the amino-acid ordering function of each template is the same.

3. Repressor hypothesis. The templates in inducible and constitutive cells are the same and do not require activation by inducers. Substances specifically inhibiting template function (repressors) are synthesized by inducible cells but not by constitutive cells. Exogenous inducers function by destroying this inhibition of enzyme synthesis.

V. EVALUATION OF THE INDUCTION HYPOTHESES

The data presented thus far do not allow much more than an intuitive preference for one of the three hypotheses over the others. Thus, the enzyme repression observed in the enzymes involved in arginine biosynthesis can equally well be explained by the inhibition of endogenous induction as by direct inhibition of template catalysis not involving an inducer. However, experiments recently reported by Pardee *et al.*[23] have done much toward clarifying the relative weight that one can place on the three alternative explanations of enzyme induction. These experiments were designed to observe the behavior of β-galactosidase synthesis in *E. coli* zygotes having a heterogenotic structure for both the z^+, z^- and the i^+, i^- pairs in an effort to determine dominance in the i^+, i^- pair. This determination is important in the evaluation of the hypotheses, since, in the generalized induction hypothesis as it has been presented, the positive and, therefore, dominant function of synthesizing an endogenous inducer is given only to the constitutive (i^-) strain, whereas in the repressor hypothesis the positive and, therefore, dominant function of synthesizing a repressor is given only to the inducible type (i^+). In the hypothesis of two template types, both inducible (i^+) and constitutive (i^-) units are given positive functions and, therefore, no dominance in the i^+, i^- pair should necessarily exist.

The characteristics of *E. coli* conjugation allow the determination of dominance in a very unique manner. The male (*Hfr*) member of the mating pair injects its "chromosome" into the female (F^-) member through a small tubule connecting the two cells. The order in time of entrance into the female of the genetic units from a given strain of male cells is unique, and apparently no appreciable amount of cytoplasm enters with these genetic units (cf. Wollman *et al.*[28] and the chapter by Lennox p. 242). Thus, the initial cytoplasmic state of the zygote is determined by the cytoplasmic state of the female before conjugation. As a consequence, it is possible to form the i^+z^+/i^-z^- zygote either by the injection of the i^+z^+ genetic units from an inducible male into an i^-z^- formed cytoplasm of a constitutive absolute negative female or vice versa—to inject the i^-z^- genetic units into an i^+z^+ formed cytoplasm. The question then asked is what is the behavior of β-galactosidase synthesis in the i^+z^+/i^-z^- zygote in each case and to what hypothesis does this behavior correspond? In answer to this question, it is convenient to first describe the behavior predicted by each hypothesis.

1. Under the form of the generalized-induction hypothesis presented here, the mating of a male i^+z^+ cell to a i^-z^- female in the absence of any exogenous inducer should yield the synthesis of β-galactosidase soon aftethe i^+z^+ units have been injected into the i^-z^- cytor plasm, since this cytoplasm should contain endogenous inducers which can activate the template resulting from the entering z^+ unit. It should be noted here that the z and i units are very closely linked[23,29] so that, within the minimum time units employable in conjugation experiments, the two units enter simultaneously. Furthermore, the synthesis of β-galactosidase should continue in the i^+z^+/i^-z^- zygote as long as this heterogenotic state remains intact.

In the reciprocal conjugation, the prediction would be the same, except that there might be expected to be a longer lag between the entrance of the i^-z^- units into the i^+z^+ cytoplasm and the first appearance of enzyme synthesis owing to the necessity of the entering i^- unit to catalyze the synthesis of the endogenous inducer in the zygote cytoplasm.

2. Under the hypothesis involving two types of templates for constitutive (i^-) and inducible (i^+) synthesis, the i^+z^+/i^-z^- zygote should yield no β-galactosidase synthesis in the absence of exogenous inducer, unless the z^+ in one "chromosome" and the i^- in the other can cooperate to yield the type of template synthesized in the constitutive β-galactosidase positive strain (i^-z^+). If this is possible, then the β-galactosidase synthesis should occur in the zygote with the same behavior, whether the i^+z^+ units are injected into a i^-z^- female or

vice versa. It should be noted that the possibility of recombination leading to i^-z^+ on the same "chromosome" is negligible owing to the closeness of the z and i units.

3. Under the repressor hypothesis for induction, the injection of a i^+z^+ unit into a i^-z^- cytoplasm in the absence of inducer should yield the synthesis of β-galactosidase soon after the injection, since initially there should be no repressor in the zygote cytoplasm, it being of the constitutive (i^-) type. However, different from the prediction of the generalized induction hypothesis, in this case one should expect the synthesis of β-galactosidase in the zygote to cease as soon as the injected i^+ unit has caused the synthesis of sufficient repressor in the zygote cytoplasm. At this time, the zygote should be phenotypically inducible rather than constitutive as it was immediately after the entrance of the i^+z^+ unit.

In the reciprocal mating, the cytoplasm of the zygote, being i^+z^+, should initially contain the repressor and should continue to synthesize repressor after the i^+z^+/i^-z^- state is established. Thus, no synthesis of β-galactosidase in absence of exogenous inducer would be expected at any time after conjugation.

Each hypothesis yields a different prediction, and, consequently, the experiment should uniquely determine which, if any, is valid. The results are entirely consistent with the repressor hypothesis. Thus, in the conjugation of i^+z^+ males with i^-z^- females in the absence of exogenous inducer, β-galactosidase synthesis can be detected within a few minutes after the entrance of the i^+z^+ genetic units. However, in the reciprocal conjugation, no enzyme synthesis could de detected in the absence of inducer, even after several hours. Furthermore, in the i^+z^+ male-to-i^-z^- female conjugation the synthesis of β-galactosidase in absence of inducer ceases about two hours after zygote formation. This is shown in Fig. 6. This cessation of synthesis is not the result of segregation of the zygote, since this event cannot be detected until two hours after cessation of synthesis. As is shown in Fig. 6, the zygotes have become inducible by the time the constitutive synthesis stops. These results are exactly those predicted under the repressor hypothesis in which i^+ was predicted to be dominant over i^-, and they are inconsistent with the two other hypotheses. These results, furthermore, offer the evidence promised earlier that the i and z units involve different cistrons—i.e., different functional genetic units.

Does this experiment then offer the death knell to the hypothesis of different templates and to the generalized induction theory? It quite effectively eliminates the former of these possibilities, but, unfortunately, the generalized induction hypothesis can be made viable again by a very slight alteration. The supposition made under this hypothesis was that the i^+ to i^- mutation involved the repair of a genetic unit necessary for the synthesis of endogenous inducer. This supposition is clearly eliminated by the experiments of Pardee, Jacob, and Monod. However, it can be assumed that, in both

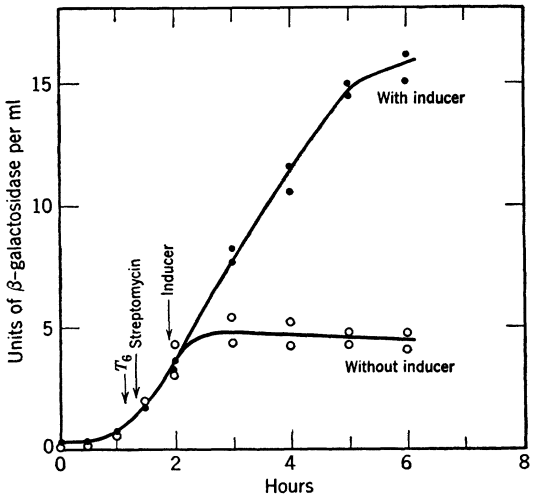


FIG. 6. Formation of β-galactosidase in zygotes from a $\sigma^+i^+z^+ \times \varphi i^-z^-$ conjugation of *E. coli*. The abscissa indicates the time after mixing the parental populations. The experiment was performed in quadruplicate. Bacteriophage T_6 and streptomycin were added at the indicated times to stop conjugation and to eliminate the σ^+. The inducer, methyl-β-D-thiogalactoside ($10^{-3}M$), was added at 115 min to two of the four cultures (filled circles), whereas the other two cultures (open circles) contained no inducer [from A. B. Pardee, F. Jacob, and J. Monod, Compt. rend. **246**, 3125 (1958)].

i^+ and i^- cells, an endogenous inducer is synthesized, but that i^+ cells differ from i^- cells in possessing an enzyme capable of destruction of this endogenous inducer. The i^+-to-i^- mutation would then result in the loss of ability to synthesize this enzyme, and i^+ would be expected to be dominant to i^-. The results of the above experiment are equally well in accord with this extension of the generalized induction hypothesis as with the repressor hypothesis. Thus, this ingenious experiment has indeed limited the choice of hypotheses drastically; it has pushed the repressor hypothesis fully into the limelight, but it unfortunately does not offer a final unambiguous solution.

Nor should one expect such an unambiguous solution as long as one is forced to deal with the complexity of an entire cell. At this level, a solution will be unique only in that it unifies and poses the answer to a larger number of problems with a lesser number of assumptions than does its alternatives. In this sense, the repressor hypothesis is the more satisfying. It yields a mechanism for feedback control of enzyme synthesis that would seem to be necessary if the cell is not to run amuck. If the phenomenon of enzyme repression becomes a general observation, particularly in biosynthetic pathways such as that found in arginine synthesis, one can account for feedback control with the use of one or less repressor substances per enzyme. However, in choosing the generalized induction hypothesis, if one wishes to maintain the explanation of feedback control, one must involve inducers and substances inhibiting induction (i.e., repressors) for each enzyme. Thus, if both hypotheses are generalized, it would seem that

feedback control is explained with a lesser number of components and consequently, assumptions, by the repressor hypothesis. On this basis, the inducible systems are seen as a peculiar form of a general repressible condition, peculiar in that they are relatively rare events of exceptionally strong endogenous repression. It is of interest to note that, should no exogenous inducer be known for such a system, it would appear as a typically negative mutant for the synthesis of a given enzyme.

If one wishes to find the unambiguous solution of enzyme induction and repression, it is the author's conviction that it will first be necessary to find a system of enzyme synthesis less complex than the whole cell. It is true that the two hypotheses pose clearly distinguishable alternatives in that one predicts the presence of an inducer in constitutive cells and the other of a repressor in inducible cells. However, it is also the strong suspicion of the author that the assay system necessary to test such exogenous inducers or repressors will require something much less complex than the whole cell. For this reason—and because resolvable subcellular systems of enzyme synthesis appear to be close at hand—the next step forward in the understanding of enzyme induction and repression will most probably attend the removal of the cell boundary from the assay system.

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