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# ENZYME INDUCTION AS AN ALL-OR-NONE PHENOMENON\*

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The phenomenon of enzyme induction (enzymatic adaptation) has been observed in a variety of micro-organisms. One of the most carefully studied examples, largely the work of Jacques Monod and his colleagues at the Institut Pasteur, is the induction of the synthesis of the enzyme  $\beta$ -galactosidase in the bacterium *Escherichia coli*.<sup>1, 2</sup>

This enzyme, not otherwise present in appreciable amounts, is formed by the bacteria when grown in the presence of lactose,  $\beta$ -galactosidase being necessary for the utilization of this sugar. A number of other galactosides also induce the formation of this enzyme in *E. coli*, including some compounds, such as thiomethyl- $\beta$ -D-galactoside (TMG), that are not split by this enzyme.<sup>3</sup> The fact that TMG is not split by the enzyme or otherwise utilized by the bacteria gives it a great advantage over lactose in kinetic studies. Thus lactose used as an inducer can also serve as an energy source, and so an increase in galactosidase content may bring about an increase in growth rate. In contrast, with inducers such as TMG, together with succinate in place of lactose as the energy source, an increase in enzyme content has no such effect. Induction under these circumstances has been called "gratuitous."<sup>1</sup>

Monod, Pappenheimer, and Cohen-Bazire<sup>1</sup> studied the kinetics of induction under gratuitous conditions. They discovered that, upon addition of inducer at a sufficiently high concentration to a growing bacterial culture, the bacteria almost immediately begin to make enzyme at the maximum rate. Since the enzyme is being made at a constant rate per bacterium, the enzyme per bacterium in the bacterial culture rises and subsequently levels off at a value determined by this rate. After one bacterial doubling, the enzyme concentration reaches 50 per cent of its ultimate value. (It reaches 63 per cent in one generation, where one generation is defined by the doubling time divided by  $\ln 2$ .)

In order to see whether individual bacteria participate equally in the synthesis of galactosidase, Benzer<sup>4</sup> studied the distribution of enzyme among the bacteria in a culture under a variety of conditions. He found that under gratuitous conditions high concentrations of inducer produce a uniform distribution of enzyme among individual bacteria as early as 5 minutes after the addition of inducer. Under these conditions the kinetics of induction of a culture represents the kinetics of the individual organism. Under conditions where the inducer is the sole carbon source, the distribution of enzyme among the bacteria is not uniform at first but becomes uniform when the culture reaches its maximum rate of enzyme synthesis.

Subsequently it was discovered in Monod's laboratory<sup>2, 5</sup> that two independent processes are usually involved in the induction of  $\beta$ -galactosidase synthesis. On the one hand, the rate of galactosidase synthesis is determined by the concentration of inducer inside the bacterium; and, on the other, the inducer is actively transported into the bacterium to give a much higher inducer concentration inside the bacterium than in the medium. The transport is accomplished by a second enzyme,

called "galactoside permease," which is also induced by TMG. When permease is present at maximum levels, the internal TMG concentration is about 100 times that in the medium.

On the basis of these facts, one must expect that at low inducer concentrations the rate of  $\beta$ -galactosidase synthesis will rise with time after the addition of inducer to the growing culture, because the internal inducer concentration increases as more and more permease is formed. One should also expect that bacteria grown in a high concentration of inducer will have a high permease content. If these "preinduced" bacteria are subsequently grown in a low external inducer concentration, they will be able to maintain a high internal inducer concentration and as a result will make both  $\beta$ -galactosidase and permease at a high rate. This is the explanation that Monod<sup>2</sup> has given for what has been called the "preinduction effect."

Under normal conditions glucose inhibits the induction of both the enzyme and the permease. However, Melvin Cohn<sup>6</sup> discovered that if the bacteria are preinduced by TMG and glucose is then added, there is no inhibition by glucose. The high permease content of the bacteria results in a sufficiently high internal inducer concentration to overcome the inhibitory effect of glucose.

We investigated the kinetics of  $\beta$ -galactosidase formation by bacteria growing at low inducer concentrations. Immediately upon the addition of inducer, the rate of galactosidase synthesis per bacterium rose linearly and continued in this way for a number of generations. It was difficult to understand this result on the assumption that each bacterium has about the same enzyme content. We were able to show that this assumption does not apply at low inducer concentrations. We discovered that at the low inducer concentrations used in these experiments the population consists essentially of individual bacteria that are either making enzyme at full rate or not making it at all. As the fraction of fully induced bacteria in the population rises, there is an increase in the *average* rate at which enzyme is produced.

This can be understood on the following basis. When inducer is added to a culture of growing bacteria, there is a certain chance, determined by the inducer concentration, that a given bacterium will produce its first permease molecule. Once a bacterium has one permease molecule, the internal inducer concentration is raised, and the probability of the appearance of a second permease molecule is increased. In this sense the induction of permease in the individual bacterium is an autocatalytic process, and, within a short time after the appearance of its first permease molecule, the bacterium becomes fully induced, synthesizing both permease and galactosidase at maximum rate. Because the transition is accomplished so rapidly, the relative number of bacteria at an intermediate state of induction is small. When a fully induced bacterium divides, both daughter cells remain fully induced. Since a constant fraction of the uninduced bacteria get their first permease molecule in each unit of time, there is an initial linear rise in the proportion of fully induced bacteria.

The rise in the proportion of the population induced would be expected to continue until the whole population is induced. However, we found that at low inducer concentrations the rise leveled off when only a fraction of the population had been induced (here called "intermediate saturation"). This fact could be ex-

plained by the subsequent observation that induced bacteria grow more slowly than uninduced. Hence at intermediate saturation there is a relative loss of induced bacteria at a rate equal to the rate of appearance of newly induced cells. Another consequence of the difference in growth rates of induced and uninduced bacteria is seen in the fact that at low inducer concentrations a fully induced culture can be maintained fully induced indefinitely, but a culture that is not fully induced, i.e., one composed of a mixture of induced and uninduced bacteria, cannot be maintained at its initial level. Although the fully induced bacteria in the mixture are maintained induced, their proportion is reduced because of the more rapid growth of the uninduced fraction.

*Technique.*—The B strain of *E. coli* was used in these experiments, all of which were performed at 37° C. and in synthetic medium at pH 7. This medium contained *M*/10 sodium succinate, *M*/30 potassium phosphate buffer, *M*/1,000 magnesium sulfate, *M*/700 sodium citrate, and *M*/50 ammonium chloride.

Many of the experiments were performed with bacteria growing in well-aerated test tubes. In other cases experiments were performed with bacteria growing in the chemostat, a continuous culture device.<sup>7</sup> The bacteria were grown with ammonia as the limiting growth factor at a generation time of 3 hours (2.1-hour doubling time). An input concentration of 20 mg./l. of NH<sub>4</sub>Cl was used, which gives a population of optical density 0.120 at 350 m $\mu$  when measured in the Beckman DU spectrophotometer.

In all cases TMG (thiomethyl- $\beta$ -D-galactoside) was used as the inducer, and  $\beta$ , galactosidase was determined by measuring the rate of which toluenized samples of bacterial culture hydrolyze the chromogenic substrate, *o*-nitrophenyl- $\beta$ -D-galactoside (ONPG).<sup>8</sup> We found in preliminary experiments a persistent variability of 10–20 per cent in enzyme activity from one toluenized sample to another. By adding 10  $\mu$ g. of sodium desoxycholate to 1-ml. samples along with 0.02 ml. of toluene and shaking for 10 minutes at 37° C., we were able to obtain reproducibility limited only by pipetting errors. Enzyme activities are expressed as the fraction of the maximum obtainable activity. Under our conditions maximum activity for a bacterial sample of optical density 0.120 is 225 m $\mu$ moles of ONPG hydrolyzed per milliliter per minute at 28° C. at pH 7 in *M*/10 sodium phosphate buffer.

It was discovered during preliminary experimentation that the rate of induction at low inducer concentrations is very much dependent on the CO<sub>2</sub> concentration.<sup>9</sup> To minimize variability due to increasing CO<sub>2</sub> production in a culture of increasing size, all cultures were aerated with air containing 4 per cent CO<sub>2</sub>.

*The Kinetics of Enzyme Formation.*—The induction of enzyme formation can be observed in the chemostat in the following way. The growth tube of a chemostat is inoculated, and the chemostat is allowed to run until the bacteria are in a steady state. At a time designated as zero, TMG is added to the reservoir and to the growth tube to the desired concentration. As a result, this concentration is from then on automatically maintained. At various times after the addition of inducer, aliquots of bacteria are removed and assayed for  $\beta$ -galactosidase activity. In this way one may observe the rise in enzyme concentration in the bacterial culture as a function of the time elapsed since the addition of inducer.

From the rise in enzyme concentration one can compute the rate, *S*, at which the bacteria make enzyme. (*S* is defined in units of enzyme made per generation by

$N$  bacteria, the number per milliliter in the chemostat.) The rate at which enzyme concentration,  $z$ , changes,  $dz/dt$ , is given by the difference between the rate  $S$  at which the bacteria make enzyme and the rate at which enzyme is diluted by the flow of nutrient liquid. (Since the flow rate in the chemostat equals the growth rate, this loss corresponds to the dilution of the bacterial mass by the formation of new mass in a growing culture.) This is expressed by

$$\frac{dz}{dt} = \frac{S}{\tau} - \frac{z}{\tau}, \quad (1)$$

where  $\tau$  is the generation time ( $\tau = \text{doubling time}/\ln 2$ ). Therefore  $S$  is given by

$$S = \tau \frac{dz}{dt} + z. \quad (2)$$

It should be noted that whenever  $S$  is constant,  $z$  will tend to become equal to  $S$ . Furthermore, whenever  $dz/dt = 0$ , the enzyme concentration is at some constant value  $z$ ; therefore, the bacteria must be making enzyme at a rate of  $z$  per generation.

When an experiment is performed at a high TMG concentration, for example  $10^{-3} M$ , one should expect on the basis of earlier work that the rate of enzyme formation will rise very rapidly to its maximum value,  $S_{\max}$ . As a result, the concentration of enzyme should rise linearly to begin with, and then more slowly as it asymptotically approaches  $S_{\max}$ . In one generation the concentration of enzyme should reach 63 per cent of its ultimate value (50 per cent in one doubling). A typical experiment at high TMG concentration is shown in Figure 1, where it is evident that, except for a short lag, the rise in enzyme concentration conforms to expectation.

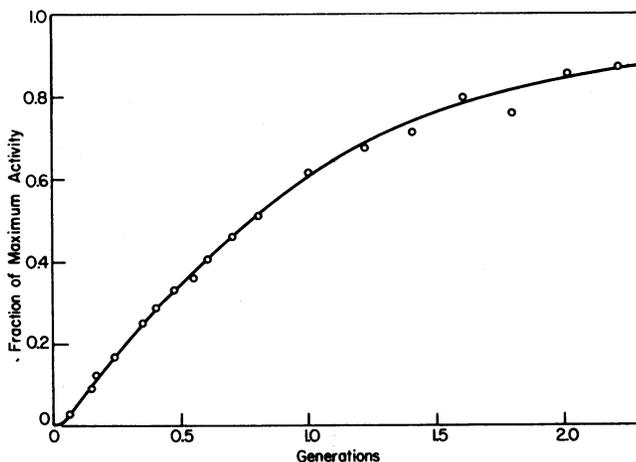


FIG. 1.—Rise in  $\beta$ -galactosidase activity following addition of  $5 \times 10^{-4} M$  TMG to a bacterial culture of optical density 0.120 growing at a generation time of 3 hours in the chemostat.

When such an experiment is performed at fairly low TMG concentrations, strikingly different results are obtained. Many generations are required before the enzyme concentration reaches 63 per cent of its ultimate value. An example

of such an experiment, with  $7 \times 10^{-6} M$  TMG, is shown in Figure 2. Following the addition of inducer, the enzyme concentration first rises more rapidly than linearly but after a generation or so rises along a straight line. After several generations of such a linear rise, the slope of the rise falls off as the enzyme concentration approaches some ultimate constant value.

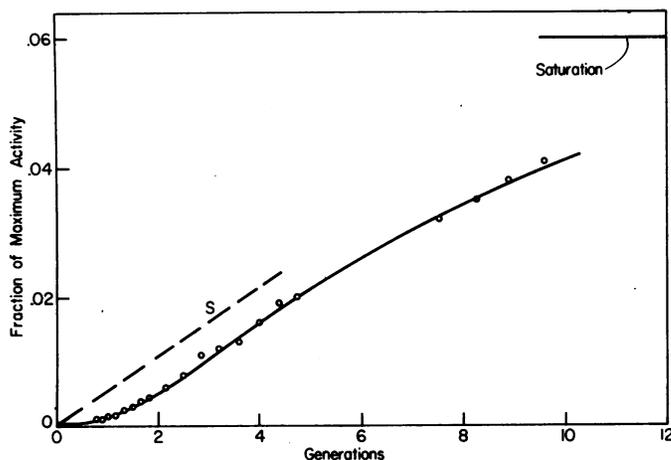


Fig. 2.—Rise in  $\beta$ -galactosidase activity following addition of  $7 \times 10^{-6} M$  TMG to a bacterial culture of optical density 0.120 growing at a generation time of 3 hours in the chemostat.

The rate,  $S$ , at which the bacteria synthesize enzyme has been computed from this rise in enzyme concentration by means of equation (2). The resulting values of  $S$  are plotted as the dotted line in Figure 2. It can be seen that the rate of synthesis rises from zero time along a straight line, paralleling the linear rise in enzyme concentration. The slope,  $K$ , of this line gives the amount by which the rate of synthesis is increased each generation.

Similar experiments were performed at a series of TMG concentrations, and it was found that with an increase in inducer concentration there is a sharp increase in the slope,  $K$ , of the straight line and an increase in the ultimate saturation value. These values are given in Table 1. It should be noted that, at higher inducer

TABLE 1  
SLOPE ( $K$ ) OF RISE IN SYNTHETIC CAPACITY AND LIMITING VALUE OF SYNTHETIC CAPACITY AFTER LONG TIMES (INTERMEDIATE SATURATION) FOR A SERIES OF INDUCER CONCENTRATIONS\*

TMG Concentration	$K$	Intermediate Saturation	TMG Concentration	$K$	Intermediate Saturation
$7 \times 10^{-6} M$	0.0051	0.0665	$9 \times 10^{-6} M$	0.0437	0.250
$8 \times 10^{-6} M$	0.0143	0.129	$10 \times 10^{-6} M$	0.0874	0.433

\* The intermediate saturation values are expressed as the fraction of the maximum rate of synthesis, and  $K$  is given in units of the fraction of maximum synthetic capacity reached per generation.

concentrations, the saturation value never exceeds the "ceiling" value,  $S_{\max}$ , the maximum capacity for making  $\beta$ -galactosidase. Furthermore, at the higher inducer concentrations  $K$  becomes very large, and the maximum rate of synthesis is reached in a very short time after the addition of inducer.

*Transfer and Maintenance Experiments.*—Preinduction with a high concentration of TMG gives a bacterial culture which continues to synthesize enzyme at maxi-

imum rate upon subsequent transfer to much lower concentrations of inducer.<sup>2</sup> We have investigated this phenomenon and find that, at certain low concentrations, the high rate of synthesis of a preinduced culture can be maintained indefinitely at a lower concentration of inducer. In fact, there are low concentrations, which we shall call *maintenance concentrations*, that will maintain full synthetic activity, despite the fact that, at these low concentrations of inducer, bacteria that are not preinduced never make enzyme at more than a negligible fraction of the maximum rate. For example, at  $5 \times 10^{-6}$  M TMG in succinate medium aerated with air plus 4 per cent CO<sub>2</sub>, a maximally preinduced culture of B strain maintains full synthetic activity indefinitely (180 generations in one experiment), and a culture of B strain that is not preinduced never attains more than 0.5 per cent of the maximum rate of synthesis.

The actual value of the concentration which may be satisfactorily used as a maintenance concentration depends on the choice of inducer and on the bacterial strain. Furthermore, the conditions under which the bacteria are growing play an important role in the effectiveness of a given concentration of inducer. In succinate medium, for example, only about one-fifth as much inducer is needed as in lactate or maltose. In addition, we find that the concentration of carbon dioxide in the medium markedly affects the response to a given inducer concentration. Unless the carbon dioxide concentration is maintained constant, an inducer concentration that is suitable for maintenance at low bacterial densities becomes a strongly inducing concentration at higher bacterial densities.

If preinduced bacteria are transferred to medium with no inducer, enzyme synthesis ceases immediately, and the enzyme present in the bacteria is diluted among the daughter cells as the bacteria divide. Between zero and maintenance inducer concentrations there is a range of concentrations where the enzyme level is not maintained because of a progressive fall in the rate at which enzyme is made. In this range of concentrations the enzyme content of the culture falls exponentially with time at a rate determined by the inducer concentration. A series of such falls in enzyme content is shown in Figure 3.

The maintenance phenomenon provides the basis for a useful technique for the determination of the synthetic capacity of a bacterial culture, especially if the synthetic capacity is changing rapidly with time. One need only transfer an aliquot of bacteria to a maintenance concentration of inducer and permit the bacteria to grow there for several generations. The rate of enzyme synthesis becomes frozen at the value it had at the time of transfer, and the enzyme concentration per bacterium in the culture soon becomes equal to the amount of enzyme made per bacterium per generation. However, when a culture induced to *less* than maximum synthetic activity is transferred to maintenance concentration, the rate of synthesis is not maintained indefinitely. There is a slow exponential fall in the rate of synthesis of the culture of about 7 per cent per generation, so that after fourteen generations the synthetic activity is down to 37 per cent of its initial value.<sup>10</sup> As a result, whenever the rate of synthesis of a culture which is less than maximally induced is measured by transfer to maintenance concentration, correction must be made for this fall. Correction must also be made for the enzyme that would have been formed in uninduced bacteria grown for the same time in that concentration of inducer.

We have used this transfer technique to observe the rise in synthetic capacity during the induction experiment shown in Figure 2. At various times during the experiment, aliquots of bacteria were withdrawn and transferred to tubes containing a maintenance concentration of inducer. The enzyme content of the transferred samples was measured after three generations of growth, at which time the enzyme content per cell should have been within 5 per cent of the rate of synthesis.

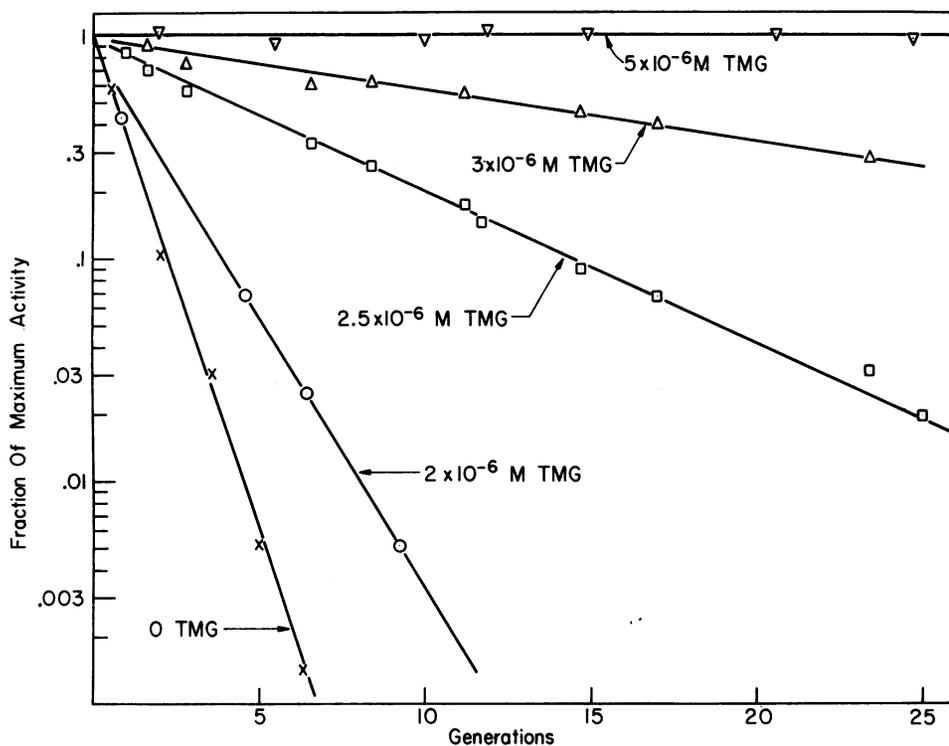


FIG. 3.—Change in  $\beta$ -galactosidase content of a bacterial culture preinduced at high TMG concentration and transferred to various low TMG concentrations. This experiment was performed by first preinducing bacteria to maximum synthetic rate by growth for several generations at  $10^{-3}$  M TMG and then inoculating them into a chemostat having  $5 \times 10^{-6}$  M TMG in the reservoir and sufficient ammonia to give an optical density of 1.20. The bacteria in this donor chemostat were maintained at maximum enzyme content and were used to inoculate the chemostats having less than maintenance inducer concentrations. This was done by making a tenfold dilution from the donor chemostat into chemostats having an ammonia concentration designed to give a bacterial population of optical density 0.120. Upon inoculation, the chemostat flow was started. The TMG concentration in the growth tube soon corresponded to that in the reservoir, since the quantity transferred from the donor chemostat was small.

These values, after being corrected as described in the preceding paragraph, were plotted as a function of the time of transfer to maintenance concentration. The results are given in Figure 4. The solid circles, which give the rate of synthesis determined in this fashion, are in good agreement with the broken line, which represents the rate of synthesis computed (by means of eq. [2]) from the rising enzyme curve.

*Cellular Distribution of Synthetic Capacity.*—Whenever kinetic experiments are

performed using bacterial cultures, the question must be raised whether the results obtained represent the events occurring within the individual cell or some average of a heterogeneous population. As stated earlier, Benzer<sup>4</sup> investigated this problem by means of a phage lysis method. He found that under gratuitous conditions of induction at high concentrations of inducer there is a uniform distribution of enzyme among the bacteria, even of the small amounts present as early as 5 minutes after the addition of inducer.

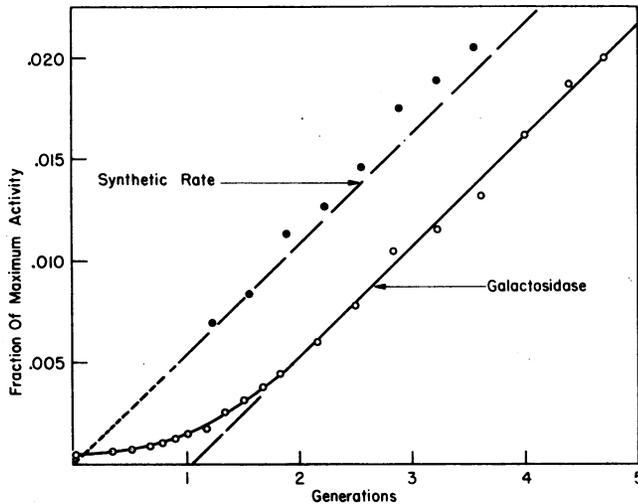


FIG. 4.—Effect of transfer to maintenance inducer concentration of bacteria taken at various times from an enzyme induction experiment such as the one shown in Fig. 2. At the indicated times, 0.25 ml. samples were withdrawn from the growth tube of the chemostat and diluted into 5 ml. of medium having excess ammonia and  $5 \times 10^{-6} M$  TMG for maintenance. The samples were aerated at  $37^\circ C.$  with air plus 4 per cent  $CO_2$  until the bacterial density equaled that in the chemostat. The  $\beta$ -galactosidase activity and density of each sample were measured. The relative enzyme activities observed were corrected by multiplying by 1.21 to correct for the fall which occurs in three generations under these conditions in samples having less than maximum activity. From each value 0.00092 was subtracted to correct for the enzyme formed in samples transferred prior to the addition of inducer. The solid circles represent the resulting corrected values, while the broken line gives the rate of synthesis computed from the rise in  $\beta$ -galactosidase in the chemostat.

Under certain conditions the maintenance phenomenon can be used as the basis of a simple technique for analyzing the distribution of the capacity to make  $\beta$ -galactosidase among individual bacteria. If single bacteria are transferred to individual test tubes containing a maintenance concentration of inducer and are grown to a population size of  $10^8$  to permit convenient assay of enzyme content, the enzyme level of a population will be maximal if the original parent bacterium of the population was induced and will be very low if the parent bacterium was uninduced. Hence, if a culture consists of a mixture of induced bacteria and uninduced bacteria, this can be demonstrated by transferring single bacteria to maintenance concentrations of inducer.

We have performed single-cell analyses by diluting a bacterial culture to a maintenance concentration of inducer and then distributing aliquots into a large number of test tubes. A dilution was chosen which would give about one bacterium per ten tubes. Upon incubation approximately 10 per cent of the tubes developed bacterial populations, and the majority of these must have been populations that descended from single bacteria.

When a single-cell analysis was performed with a culture at maximum synthetic capacity, all the populations derived from single bacteria under maintenance conditions were found to have maximum enzyme levels. However, when uninduced bacteria were distributed into tubes containing maintenance concentration of inducer, all the populations obtained had less than 1 per cent of the maximum activity.

Distribution experiments were then performed with bacterial populations at a series of intermediate rates of synthesis. In one case a population was induced at a low concentration of TMG to a saturation value of about 30 per cent of maximum. When the population was diluted into a maintenance concentration, 30 per cent of the cultures grown from single bacteria were found to have maximum enzyme content, while 70 per cent had only very small amounts. Similar experiments performed with cultures at 10 per cent of maximum synthetic activity gave clones of which 10 per cent were fully induced and 90 per cent were uninduced. Furthermore, cultures examined during the course of a rise in synthetic activity, like the one shown in Figure 2, were found to consist of mixtures of induced and uninduced bacteria, and the induced fraction of the population corresponded to the amount of synthetic activity present.

These experiments show that under the experimental conditions being discussed a rate of enzyme synthesis less than maximum arises from the fact that the population is heterogeneous in its capacity to make enzyme, some individuals making enzyme at maximum rate and the remainder making essentially none.

*Intermediate Saturation.*—A series of experiments was then performed that provided an explanation for two puzzling aspects of the induction kinetics already noted. In the first place, at the lower inducer concentrations the rise in synthetic activity does not continue until the whole population is induced; instead, it comes to some intermediate saturation level that is less than the maximum found at high inducer concentrations. At an intermediate saturation level the culture is composed partly of bacteria making enzyme at the maximum rate and partly of bacteria making none. Why do the uninduced bacteria remain uninduced? Second, why is it that cultures at maximum activity can be maintained indefinitely by maintenance inducer concentrations, while cultures at less than maximum activity are not maintained and slowly lose synthetic activity?

The answer to these questions is provided by the observation that induced bacteria grow more slowly than uninduced. The existence of a difference in growth rate was established in two ways. In the first method the growth rates of both induced and uninduced bacteria were measured at a series of inducer concentrations by observing the increase in number of bacteria with time. The results, given in Table 2, show that induced bacteria grow more slowly than uninduced bacteria and that induced bacteria grow more slowly the higher the concentration of inducer.

The difference in growth rate between induced and uninduced bacteria was also

demonstrated with reconstruction experiments in the chemostat, employing known mixtures of induced and uninduced bacteria. Two chemostats were set up with a maintenance inducer concentration in the medium. One was inoculated with

TABLE 2  
GENERATION TIMES OBSERVED WHEN A MAXIMALLY INDUCED CULTURE OF B  
WAS INOCULATED INTO MEDIUM HAVING INDICATED TMG  
CONCENTRATIONS AND EXCESS AMMONIUM CHLORIDE\*

TMG Concentration	Generation Time (Hours)	TMG Concentration	Generation Time (Hours)
0	$2.26 \pm 0.07$	$5 \times 10^{-5} M$	$2.50 \pm 0.07$
$5 \times 10^{-6} M$	$2.38 \pm 0.07$	$5 \times 10^{-4} M$	$3.17 \pm 0.10$

\* Uninduced bacteria growing in the absence of TMG have a generation time of  $2.17 \pm 0.07$  hours.

bacteria preinduced to maximum synthetic rate, while the other received 20 per cent of such maximally induced bacteria and 80 per cent of uninduced bacteria. The results, given in Figure 5, show that the culture containing 100 per cent in-

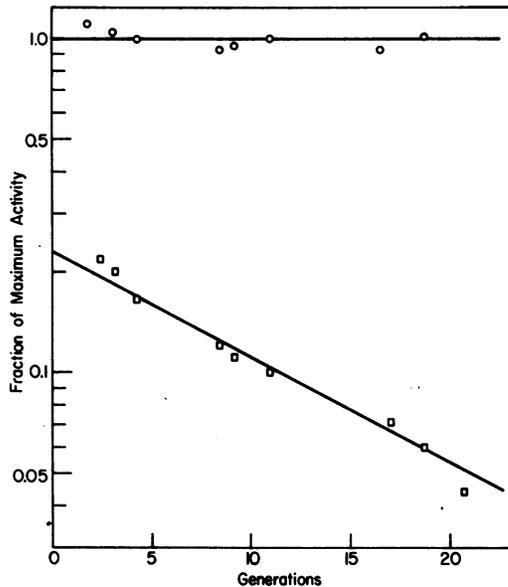


FIG. 5.—Two bacterial cultures were grown in chemostats with  $5 \times 10^{-6} M$  TMG in the medium. Initially, one (indicated by circles) was composed entirely of induced bacteria, while the other (indicated by squares) was composed of 20 per cent uninduced bacteria.

duced bacteria was maintained with no decrease in enzyme level, while in the chemostat containing the mixed population the enzyme level fell exponentially by about 7 per cent per generation. This fall in the enzyme level must be attributed to displacement of the induced bacteria by the more rapidly growing uninduced organisms.

The phenomenon of intermediate saturation can readily be understood once the difference in growth rates is established. In a typical induction experiment like the

one shown in Figure 2, the number of induced bacteria rises until the number of bacteria becoming newly induced equals the number lost as a result of the slower growth of the induced organisms. The saturation value that is ultimately reached is given by either

$$\frac{S}{S_{\max}} = \frac{K}{1 - \alpha_1/\alpha} \quad \text{for } K + \alpha_1 < \alpha \quad (3)$$

or

$$\frac{S}{S_{\max}} = 1 \quad \text{for } K + \alpha_1 \geq \alpha, \quad (4)$$

where  $\alpha$  is the growth rate constant of the uninduced bacteria and  $\alpha_1$  that of the induced bacteria. Furthermore, since the growth rate of induced bacteria decreases with increase in inducer concentration (Table 2), it is possible to understand why the intermediate saturation level does not increase in proportion to  $K$  (Table 1).

The slower growth of the induced organisms also explains why the enzyme concentration slowly falls when a culture at less than maximum synthetic activity is transferred to maintenance inducer concentration. At this concentration the number of bacteria becoming newly induced is negligible. As a result, the enzyme level falls by about 7 per cent per generation as the induced bacteria are displaced by the uninduced.

*Discussion.*—At the cellular level the induction of  $\beta$ -galactosidase is an "all-or-none" phenomenon, since bacterial populations grown at low concentrations of inducer are composed of bacteria which are either uninduced or fully induced. The kinetics of induction that we have observed therefore reflect changes in the relative number of bacterial cells synthesizing  $\beta$ -galactosidase. In an experiment of the kind illustrated in Figure 2, for example, the rate of enzyme synthesis rises linearly, because there is a linear rise in the fraction of the population in the induced state.

Such a linear rise in the induced fraction of the population must be interpreted in the following way. In the presence of inducer there is a constant probability, determined by the inducer concentration, that a cell will become induced in each generation. Once a bacterium is induced, all its progeny will be induced, since the concentration of inducer in these experiments exceeds the maintenance value. On this basis the rise will continue until the entire population is induced, unless the rate of induction is small compared to the selection which results from the lower growth rate of induced bacteria. In this event an intermediate saturation value will be reached where only a fraction of the population is induced.

The "all-or-none" character of the  $\beta$ -galactosidase induction system in *E. coli* and the inheritability of the induced state at low inducer concentrations can be understood in terms of the functioning of the inducible galactoside permease which concentrates inducer. When an inducer is added to a growing culture of bacteria, permease synthesis will be initiated at a rate determined by the concentration of inducer in the medium. At low concentrations of inducer, however, the rate of permease synthesis in uninduced bacteria may be so low that the probability

of a bacterium making a single permease molecule during its lifetime is small. Once a bacterium has a permease molecule, there will be an increase in the internal inducer concentration which will increase the probability that a second permease molecule will be formed in this bacterium. (That permease synthesis increases rapidly with increase in inducer concentration can be seen in Table 1, which shows the sharp increase of the rate of induction,  $K$ , with increase in external concentration of inducer.) The presence of two permease molecules further increases the rate of permease formation, and in this way there should be an autocatalytic rise in the permease content of this bacterium to some maximum. Once the bacterium has maximal permease, it and its progeny will be induced indefinitely, since the concentration of inducer in the medium is greater than maintenance concentration. Therefore, as more bacteria receive their first permease molecule, the fraction of induced bacteria in the population increases. In this model, the slope,  $K$ , of the linear rise gives the probability of appearance of the first permease molecule in an uninduced bacterium and is therefore determined by the external inducer concentration.

The reason for considering formation of the first permease molecule as the critical step is the following. A linear rise lasting for several generations means that the probability of a bacterium becoming induced is constant in time; hence the transition from uninduced to induced is the consequence of a single random event. This event must be the achievement of some critical threshold of permease which assures a rise in permease to its maximum. The fact that the linear rise begins at zero time suggests that this threshold is a single permease molecule.

The model also explains the maintenance experiments. Thus it is quite possible that at the low concentrations of inducer used in maintenance experiments the threshold number of permease molecules needed to drive a bacterium to maximum induction may have a value greater than 1. Nevertheless, if the number of permease molecules at maximum is large compared to the threshold, there is a high probability that, on division of a fully induced bacterium, each daughter cell will receive a sufficient number of permease molecules to assure maximal induction by the maintenance concentration of inducer. Indeed, the fact that a maximally induced culture can be maintained maximally induced for many generations shows that the chance of a bacterium becoming uninduced under these conditions is very small. Were any uninduced organisms to appear, they would be selected for by their more rapid growth and would bring about a reduction in the rate of galactosidase synthesis of the culture.

Another phenomenon that can be interpreted with the present model is the exponential fall in enzyme content that occurs upon transfer of an induced culture to a concentration which is less than maintenance (e.g., Fig. 3). The single-cell analyses show that in these cases the exponential fall in enzyme content corresponds to an exponential disappearance of induced bacteria from the population. An exponential disappearance means that there is a constant chance that a bacterium will become uninduced each generation. Once uninduced, this bacterium and all its progeny necessarily remain uninduced at these low inducer concentrations.

A constant chance of becoming uninduced can be explained by assuming that permease molecules are randomly divided among the two daughter cells. With random division there will be a certain chance that upon cell division a daughter cell

may get less than the threshold number of permease molecules needed at these very low inducer concentrations to assure induction. The chances of a cell getting less than a threshold number will be increased if this threshold is increased, as it may very well be at these very low inducer concentrations. In addition, the chances of a cell getting less than enough would be increased if the number of permease molecules present in the induced fraction of the population were decreased. Such a decrease might well be expected at these very low inducer concentrations.

*Conclusion.*—The induced synthesis of  $\beta$ -galactosidase at low concentrations of inducer bears a close resemblance to the phenomenon of mutation (in the sense of a chromosomal change). In the case of mutation a cell is either mutant or wild type; in the case of enzyme induction a bacterium is either fully induced and makes  $\beta$ -galactosidase at maximum rate or is uninduced and makes no  $\beta$ -galactosidase. All the offspring of a mutant bacterium are mutants; all the progeny of an induced bacterium are induced, as long as maintenance inducer is present. Bacteria undergo mutation as the result of some random single event; likewise, uninduced bacteria make the transition to the induced state as the result of a random single event.

However, unlike mutation, the induction system requires the continued presence of a low concentration of inducer to maintain the distinction between the induced and uninduced states. At high concentrations of inducer all the bacteria become induced, while, in the absence of inducer, the entire population becomes uninduced.

The genetic-like behavior of the state of induction can be explained by the concentration within the bacteria of inducer by a mechanism whose formation is also induced by the presence of inducer. Inducible transport mechanisms of this kind may exist for a variety of substances which enter the cell, and, as a result, careful distinction must be made between mutation and induced changes. In any event, the existence of induced inheritable changes of the kind described here raises the possibility that some differences which arise in a clone of organisms may be the result of changes in cellular systems other than the primary genetic endowment of the cell.

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<sup>1</sup> J. Monod, A. M. Pappenheimer, and G. Cohen-Bazire, *Biochim. et Biophys. Acta*, **9**, 648, 1952.

<sup>2</sup> J. Monod, in *Enzymes: Symposium of the Henry Ford Hospital* (New York: Academic Press, Inc., 1956), pp. 1-27.

<sup>3</sup> J. Monod, G. Cohen-Bazire, and M. Cohn, *Biochim. et Biophys. Acta*, **7**, 585, 1952.

<sup>4</sup> S. Benzer, *Biochim. et Biophys. Acta*, **11**, 383, 1953.

<sup>5</sup> H. V. Rickenberg, G. N. Cohen, G. Buttin, and J. Monod, *Ann. Inst. Pasteur*, **91**, 829, 1956.

<sup>6</sup> M. Cohn, in *Enzymes: Symposium of the Henry Ford Hospital* (New York: Academic Press, Inc., 1956), pp. 41-46.

<sup>7</sup> A. Novick and L. Szilard, *Science*, 112, 715, 1950; A. Novick, *Ann. Rev. Microbiol.*, 9, 97, 1955; J. Monod, *Ann. Inst. Pasteur*, 79, 390, 1950.

The chemostat maintains by continuous dilution a culture of bacteria growing indefinitely at constant density and under constant conditions. There is a growth tube which contains the growing population of bacteria, and there is a reservoir from which nutrient is fed into the growth tube in such a fashion that the contents of the tube are diluted at a rate equal to the bacterial growth rate. The bacterial growth rate is determined by the low concentration in the growth tube of some limiting growth factor. A similar apparatus, called the "bactogen," has been described by Monod. The chemostat is useful for kinetic studies because the concentrations of all chemical substances in the growth tube remain constant indefinitely, and, as a result, the bacteria remain in a constant physiological state.

<sup>8</sup> J. Lederberg, *J. Bacteriol.*, 60, 381, 1950.

<sup>9</sup> The rate of induction, *K* (to be described later), increases roughly linearly with CO<sub>2</sub> concentration up to as high as 10 per cent CO<sub>2</sub>. This phenomenon will be described in a later publication.

<sup>10</sup> This fall is discussed in the section on "Intermediate Saturation."

## THE ISOLATION OF LYSOLECITHIN FROM HUMAN SERUM\*

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The phosphorus-containing fraction of lipid extracts of human serum has been reported to contain lecithin, sphingomyelin, phosphatidyl ethanolamine, phosphatidyl serine, and plasmalogen. The techniques employed for the isolation of the individual phospholipid components have usually been laborious and have often been unreliable. An improved method of separation using adsorption chromatography on silicic acid columns or on silicic acid-impregnated filter paper has been recently reported by Lea, Rhodes, and Stoll.<sup>1</sup> With a modification of this procedure, an additional phosphorus-containing component of a lipid extract of human serum has been isolated, which, by staining properties, chromatographic mobility, chemical analysis, and hemolytic activity, appears to be lysolecithin.

### MATERIALS AND METHODS

The serum used in these studies was either pooled hospital patients' sera refrigerated for 1 day prior to use or normal sera processed within 1 or 2 hours after withdrawal.

*Extraction.*—The serum was added dropwise with shaking to 15 times its volume of a 1:1 (v/v) mixture of methanol and chloroform. After standing for 1 hour, the mixture was filtered and the filtrate emulsified with an equal volume of distilled water. Following centrifugation, the upper layer was discarded and the bottom layer taken to dryness in a rotary vacuum evaporator at a maximum temperature of 60° C. This extract was stored *in vacuo* at -30° C., usually for not more than 1 or 2 days.

*Chromatography.*—A modification of the method of Lea, Rhodes, and Stoll<sup>1</sup> was employed. The chloroform used (Fisher, A.C.S.) was washed with water and filtered, and 2 per cent methanol (v/v) was added as a preservative. The silicic