INDUCED SYNTHESIS OF ENZYMES IN BACTERIA ANALYZED AT THE CELLULAR LEVEL

by

S. BENZER*

Service de Physiologie microbienne, Institut Pasteur, Paris (France)

INTRODUCTION

The kinetics of the induced synthesis of enzymes ("enzymic adaptation") in microorganisms is usually studied by measurements upon cultures containing large numbers of cells; an inducing substance is added and the consequent appearance of enzyme in the culture is followed. Such measurements of the overall activity of the culture do not reveal whether or not all cells participate equally and simultaneously in the synthesis of enzyme. A knowledge of this factor is critical, however, for the proper interpretation of the kinetics of enzyme formation.

Consider, for example, that a given cell might synthesize its maximum amount of enzyme in an abrupt fashion, this transition occurring at random times for particular cells. In such a case, the average measurement on the culture, indicating a gradual rise in enzyme level, would bear little relation to the events in the enzyme-forming system of each cell. One is not authorized to extrapolate the average kinetics to the cellular level unless a uniform behavior of the population can be demonstrated[3,13,14,10].

In this paper, a method is described for determining the cellular distribution of an enzyme in a population of bacteria. This method is based upon the special relationship between a bacteriophage and its (individual) host cell, which permits the use of phage as a discriminating device. Application of this technique to the induced synthesis of β-D-galactosidase in *E. coli* reveals that, under certain conditions, synthesis of this enzyme proceeds uniformly in all the cells of a culture and therefore the average kinetics applies at the cellular level. Under other commonly employed conditions, however, a high degree of heterogeneity occurs, so that the average kinetics does not represent the course of enzyme synthesis within individual cells.

Mode of attack on the problem

Infection of a bacterium by phage blocks enzyme synthesis, at least in certain cases. Also, the reproduction of phage in a bacterium, culminating in lysis of the cell, requires the active metabolism of the host. Thus, if a phage-infected cell is placed under conditions where a given intracellular enzyme is indispensable for metabolism, the

* During this work the author was on leave of absence from Purdue University, as a Fellow of the Fulbright Commission and the American Cancer Society.

Present address: Biophysical Laboratory, Departments of Biological Sciences and Physics, Purdue University, Lafayette, Indiana, U.S.A.

References p. 393.

development of the phage will be dependent upon whether the enzyme was present *before* infection[8,16].

For example, *E. coli* B cannot utilize lactose unless it contains galactosidase. To test for the presence of this enzyme in a population of *E. coli* B, the cells may be infected with phage and placed in the presence of lactose as sole source of carbon and energy. In a cell that contains no enzyme, a curious deadlock occurs: the phage blocks synthesis of the enzyme, thus causing the development of the phage itself to be prevented[8]. In a cell containing enzyme, the rate at which phage development proceeds, and hence the time at which the cell lyses, should depend upon its content of enzyme. If so, the phage can serve as an instrument for discrimination between cells containing different amounts of enzyme.

Heterogeneity of the culture in regard to enzyme content can be tested for in a *qualitative* way by observing the shape of the curve of optical density as a function of time, or by microscopic observation of the lysis of individual cells.

The analysis may be made *quantitative*, however, by measurements of the enzyme released into solution by the lysed cells at various stages during the lysis of the culture. This is possible *if*: a. the amount of enzyme in a cell does not change during phage development; b. lysis occurs sooner for cells of higher enzyme content; c. the enzyme is released undestroyed into the medium when the cell lyses, and is active in solution. The preliminary experiments below justify these assumptions in the case of the system here studied.

MATERIALS AND METHODS

Bacterium. Escherichia coli, strain B.

Phage. φII, described by MONOD AND WOLLMAN[8].

Enzyme. The term enzyme in this paper refers to the β-D-galactosidase ("lactase") of *E. coli*[1,6,7].

Synthetic medium. KH_2PO_4 1.4 g; $(NH_4)_2SO_4$ 0.2 g; $MgSO_4.7H_2O$ 0.2 g; $CaCl_2$ 0.1 g; $FeSO_4.7H_2O$ 0.0005 g; KOH to pH 7.0; distilled H_2O to 1000 ml. The carbon source added in each experiment is specified in the text. In all cases, an excess of nitrogen source is present. This is a modification of medium "56" of MONOD, COHEN-BAZIRE AND COHN[9], the phosphate and ammonium sulfate concentrations being reduced by a factor of ten to facilitate adsorption of the phage. (In the preparation of phage lysates, however, the full concentrations are used, in order to obtain high phage yields.)

Temperature is 37° C in all experiments, unless specified otherwise.

Cultures of 100 ml volume are grown in 1000 ml conical flasks, aerated by shaking, in synthetic medium plus a (specified) carbon source. The inoculum is taken from an overnight culture in which growth has been limited by depletion of the same carbon source. The growth rate is 1.0 doubling per hour on glucose or lactose and 0.7 per hour on lactate.

Optical density is measured with the Meunier densitometer, using blue light. One (arbitrary) unit of optical density corresponds to approximately $4 \cdot 10^6$ cells per ml.

Enzyme activity is measured by the rate of hydrolysis of *o*-nitrophenyl β-D-galactoside (NPG)[1,6]. The enzyme activity per ml is determined by the rate of increase of optical density at 420 mμ (owing to the liberation of *o*-nitrophenol) as measured in the Beckman spectrophotometer at 28° C pH 7.0. One unit of activity corresponds to one millimicromole of NPG hydrolyzed per minute. Enzyme concentrations are expressed in units per ml. The measurements are made in the synthetic medium plus 1/10 *M* NaCl and 1/400 *M* NPG. In this solution, the enzyme is saturated with NPG, activation by sodium is complete, and competitive inhibition of the hydrolysis of NPG by the inducers used is negligible[9].

The activity of intact cells observed by this method corresponds to only a small fraction (1/20) of their actual enzyme content[6]. However, after shaking the cells with a few drops of toluene at 37° C for twenty minutes, the full activity is observed. The activity observed for extracellular enzyme is unaffected by toluene. Therefore, *total activities* for a suspension of intact or partially lysed cells are measured after toluenization, but centrifuged samples are measured for *supernatant activity* (*i.e.* enzyme released by lysis without toluenization). The *specific activity* of a culture is defined as the ratio of total activity to optical density[1].

References p. 393.

Phage stocks are prepared by lysis of *E. coli* B with phage φII, according to MONOD AND WOLLMAN[8]. Cells at a concentration of 10^9 per ml, growing rapidly in glucose, are infected by adding a similar number of phage particles. After completion of lysis, bacterial debris is removed by centrifugation, the lysate is inoculated heavily with cells of a strain (ML) resistant to the phage, incubated (with shaking) overnight to eliminate available carbon sources, and the bacteria reduced to a negligible number by centrifugation. The phage titer obtained is approximately 10^{11} infective particles per ml.

Infection of cultures is done with an input of approximately fifteen phage particles per bacterium. About two thirds of the particles become adsorbed (as determined by centrifuging the infected culture and plating the supernatant for plaque count) in fifteen minutes, giving an average multiplicity of infection of around ten.

PRELIMINARY EXPERIMENTS

Blocking of induced enzyme synthesis by phage infection

MONOD AND WOLLMAN[8] had shown that infection of "unadapted" *E. coli* by phage φII inhibited "adaptation" to lactose, as judged by the crude criteria of lysis and rate of respiration. The effect of phage infection upon formation of galactosidase is shown in Fig. 1. Within a few minutes after the addition of phage (some time is necessary for adsorption to the bacteria) further enzyme production is blocked. This holds true regardless of the level of activity before infection. The enzyme level is "frozen", neither increasing nor decreasing during the subsequent growth of phage. The increase in optical density of a growing culture is likewise halted upon infection.

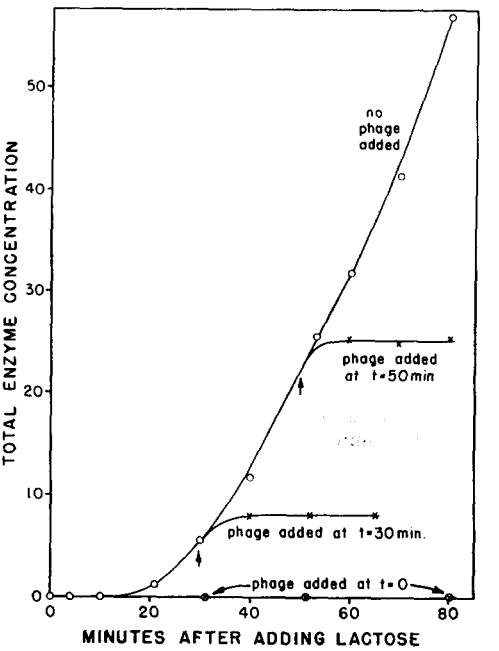

Fig. 1. Inhibition of enzyme synthesis by phage infection. Uninduced cells are prepared by growth on glucose. Twenty minutes after growth has ceased due to exhaustion of the glucose, lactose (0.1 %) is added as inducer-substrate ($t = 0$). The effect is shown of infecting the culture with phage at various times. Enzyme formed given in units per ml.

This effect is not characteristic of *all* phages. JACOB[4,5] showed that infection of *Pseudomonas pyocyanea* by a *temperate* phage fails to block completely bacterial growth or synthesis of glucozymase, while SIMINOVITCH AND JACOB[12] observed that *E. coli* K12 could still synthesize β-galactosidase during the induced development of phage λ.

Dependence of lysis time upon specific activity

The use of phage as a discriminating device is based upon the idea that if the infected cell is placed in a medium containing the enzyme substrate as sole carbon and energy source, the rate of metabolism, and hence the time required to reach lysis, will depend upon the amount of enzyme in the cell. JACOB[5] has observed such an effect when infected bacteria (*Pseudomonas pyocyanea*) are "fed" at rates controlled by fixed concentrations of *extracellular* enzyme. The existence of such a dependence for cultures

References p. 393.

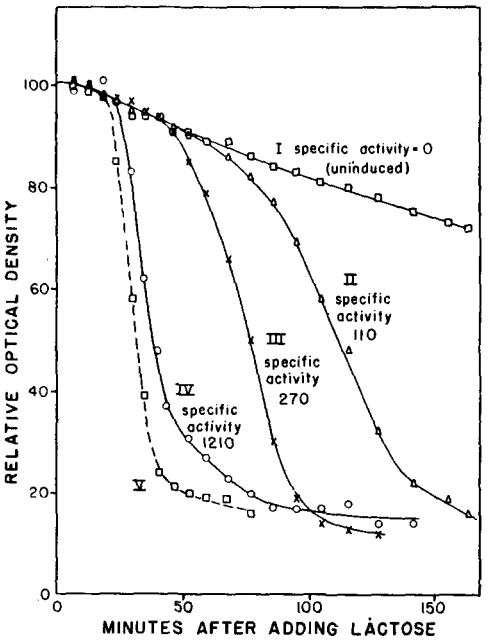

Fig. 2. Lysis of phage-infected cultures of various specific enzyme activities. Cultures induced to various degrees of specific activity are incubated in the absence of carbon source for 20 minutes, then phage is added. After 15 minutes for adsorption of the phage, lactose (0.1 %) is added at $t = 0$ (curvesI–IV). In curve V, glucose instead of lactose is added; since galactosidase is unnecessary for the utilization of this substrate, this curve is equivalent to one for a culture having unlimited enzyme content and utilizing lactose.

Liberation of enzyme by lysing cells

In a growing culture of enzymically active *E. coli* B no detectable extracellular (supernatant) enzyme is present. Fig. 3 illustrates the rise of extracellular activity as the culture lyses after infection with phage. The initial small quantity of enzyme liberated before the addition of substrate is due to lysis from without of a few cells, and is subtracted from the data hereafter presented. It will be observed that the total activity is undiminished by lysis and that most of the enzyme is recovered in the supernate; the remainder is presumably due to the failure of a fraction of the cells to lyse. Lysis by phage appears to be an efficient method for extracting cellular enzymes.

References p. 393.

of *E. coli* induced to various levels of intracellular galactosidase is shown in Fig. 2.

The exact time at which a cell of particular enzyme content lyses is affected by other physiological variables. Therefore, it cannot be expected that the cells will lyse strictly in the order of their enzyme content. This randomizing effect is undesirable, since it makes the observed enzyme distribution appear more uniform than the true one. It can be minimized by using very active cells.

The dependence of time of lysis on enzyme content breaks down, of course, at very high enzyme levels, the rate of phage development becoming limited by other factors.

It will be observed from Fig. 2 that even cells devoid of enzyme do lyse to some extent. This "lysis from without" (DELBRÜCK[2]), the mechanism of which is not known, becomes increasingly marked as the ratio of phage particles to bacteria is increased. Since lysis from without is independent of specific activity, it does not contribute to the desired discrimination and its effect is also to make the observed distribution appear more uniform than the true one. This effect is minimized by using the smallest multiplicity of infection required to reduce the fraction of uninfected cells to a negligible value.

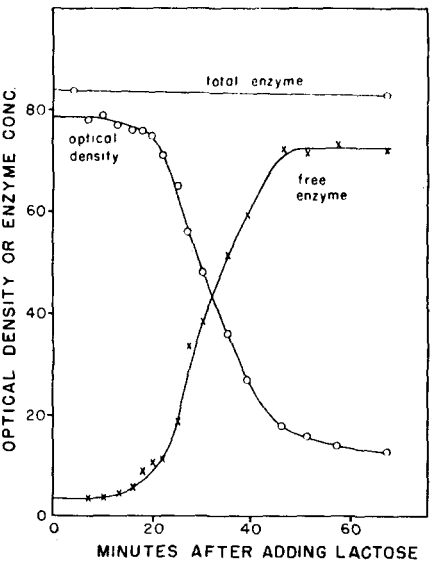

Fig. 3. Liberation of intracellular enzyme of phage-infected cells by lysis. *Free enzyme* is measured in the supernate of a centrifuged sample. *Total enzyme* is measured on an uncentrifuged (toluene-treated) sample. Enzyme concentration expressed as units per ml.

The constancy of total enzyme provides a convenient test for the completeness of infection in a given experiment, without the necessity of making plate counts. If any significant number of uninfected or phage resistant bacteria are present, the total activity rises with time.

The results of these preliminary experiments appear to justify the proposed method.

PROCEDURE FOR ANALYSIS OF ENZYME DISTRIBUTIONS

The following steps are taken in order to analyze the cellular distribution of enzyme in a culture (previously induced under specified conditions):

1. The culture to be analyzed is agitated at 37° C for 30 minutes in synthetic medium containing a source of nitrogen, but devoid of carbon and energy source, in order to starve the cells.

2. Phage is added, and becomes adsorbed to, but does not reproduce in the starved bacteria. The multiplicity of infection (*i.e.* ratio of *adsorbed* phage particles to cells) is sufficient to insure that very few bacteria are uninfected.

3. After 15 minutes allowed for phage adsorption, lactose (to final concentration of 0.1%) is added.

4. At various times while the culture lyses, samples are removed and chilled in an ice bath. Chilling does not completely stop lysis but slows its rate by a factor of twenty thus making negligible the time required for carrying out the further operations.

5. Each sample is divided in two. One part is measured for optical density. The other is centrifuged (in the cold) to sediment the unlysed bacteria, and the supernate is collected. The supernate is (later) assayed for enzymatic activity.

6. For each sample, the increase of activity in the supernate is plotted against the decrease in optical density (compared with their values at the time at which lactose was added).

The graph of enzyme liberated against decrease in optical density (proportional to the number of cells lysed) reflects the distribution of enzyme within the population. The slope of the curve, being the ratio of enzyme concentration to optical density, represents the specific activity of that component of the population which is lysing. Thus, the initial and final slopes represent the maximum and minimum specific activities present in the culture, and the entire distribution can be found from the curve.

Fig. 4 illustrates the form of the curve for several hypothetical distributions. If all cells contain equal amounts of enzyme, the graph is linear. For an all-or-none distribution, the curve consists of two intersecting straight lines. Cells which are devoid of enzyme eventually lyse also, since the enzyme

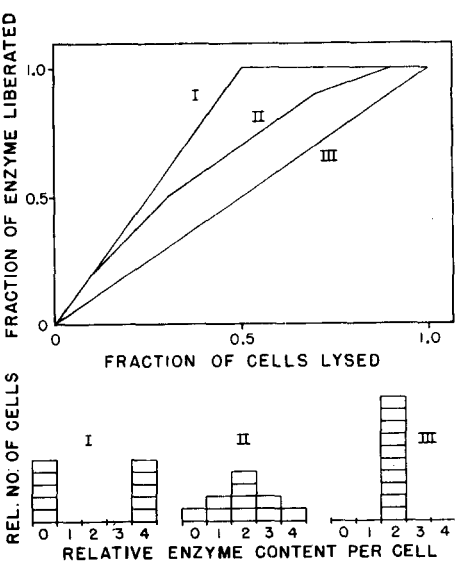

Fig. 4. Below: three hypothetical distributions of enzyme in populations of bacteria. Above: The curves expected from analysis of these populations by the phage procedure.

References p. 393.

released from early-lysing cells hydrolyzes lactose, liberating glucose which can be utilized by all cells. However, no further rise in supernatant enzyme concentration results from the lysis of these cells.

Test of the method on artificial mixtures of cells

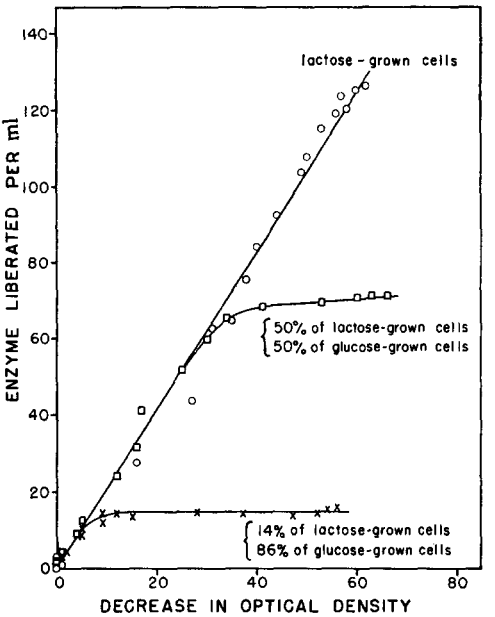

Fig. 5. Resolution of artificial mixtures of induced (lactose-grown) and uninduced (glucose-grown) cells by the phage procedure.

The validity of the method can be tested on artificial mixtures of enzymically active and inactive cells. Fully active (lactose-grown) and inactive (glucose-grown) cells are mixed, in known proportions, and the above-outlined method applied for analysis (Fig. 5).

The heterogeneity of the mixtures is very clearly brought out by the composite curves obtained for them. The limitations on the resolution of the method are visible in the rounding of these curves, which would break sharply if the method were perfect. Note that the lactose-grown culture by itself gives a straight line, indicating a uniform distribution of enzyme in such cells or, at least, that there is enough enzyme in all cells so that the quantity of enzyme is not a limiting factor in the time required for lysis. (That the distribution is indeed uniform will be seen below, under "de-induction".)

DISTRIBUTION OF ENZYME AT SUCCESSIVE STAGES FOLLOWING INDUCTION

The manner in which individual cells of a culture participate in the over-all synthesis of an induced enzyme might be expected to depend upon the conditions under which induction is brought about. For example, in many experiments on "adaptation" the substance that is used to induce enzyme synthesis is the only external metabolic source of carbon and energy, and cannot be utilized except by virtue of the enzyme. Under these conditions, the synthesis of enzyme must depend upon traces of enzyme already present, or upon internal bacterial reserves. Any cell that has a start over the others will gain increasingly, and the population of cells may be expected to develop an exaggerated heterogeneity in respect to enzyme content at a given time.

In induction under "conditions of gratuity"[10] the metabolic requirements of the cells are supplied by substrates which are available without the enzyme, so that all cells can metabolize rapidly, regardless of enzyme content. Addition of an inducer (ideally, a non-utilizable substance) initiates synthesis of the desired enzyme, which can proceed, at any time, independently of the amount of enzyme previously formed.

In the following experiments, the cellular distributions of enzyme are determined in cultures induced under the two different sets of conditions described above.

References p. 393.

Induction by inducer carbon source

The cells are grown in glucose until growth stops owing to exhaustion of the glucose, then lactose is added as inducer and source of carbon.

In Fig. 6A the optical density and (average) enzyme content are given as functions of time after addition of the inducer-substrate. There is a lag of around 15 minutes before considerable amounts of enzyme appear. In Fig. 6B the *increase in enzyme* is plotted against the *increase in optical density*, in the manner of MONOD, PAPPENHEIMER AND COHEN-BAZIRE[11]. The shape of this curve indicates that the ratio of enzyme synthesis to total *new* growth is initially greater than its final value.

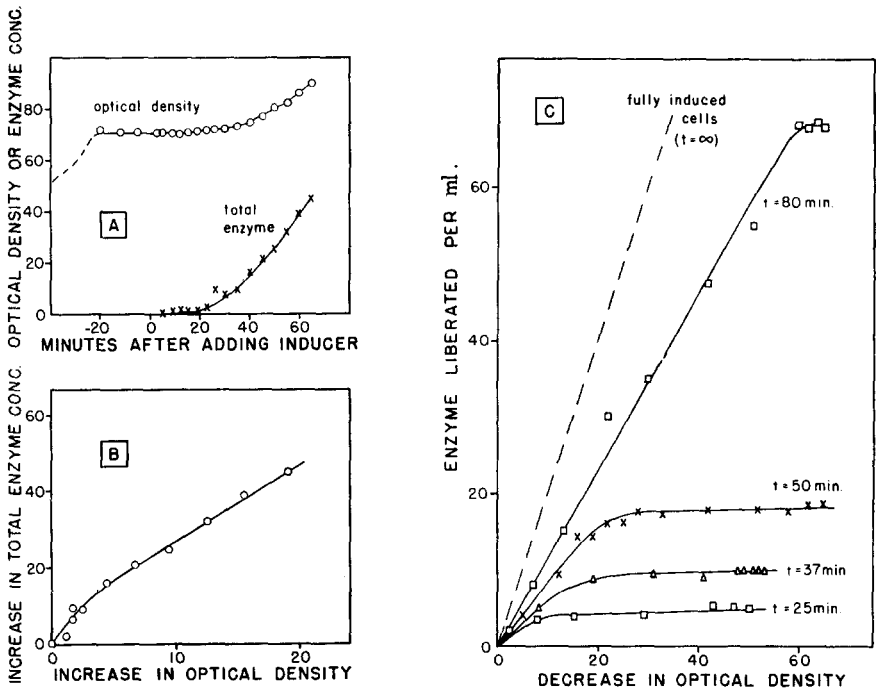

Fig. 6. Induction by inducer-substrate (lactose). Uninduced cells are prepared by growth on glucose. Twenty minutes after growth is arrested due to exhaustion of the glucose, lactose (0.1 %) is added ($t = 0$).

A. Enzyme formed and optical density as functions of time.
B. Relation between enzyme formed and *new* growth following addition of the inducer-substrate.
C. Analysis, by the phage procedure, of cultures induced for various times under these conditions. Enzyme concentration = units per ml.

By taking samples at various times and analyzing each by the phage procedure (outlined above), one arrives at the results shown in Fig. 6C. At early times, a few cells contain most of the enzyme. As time goes on the fraction of enzyme-containing cells increases, the culture eventually becoming homogeneous. Clearly, in this case, the average kinetics for the culture *does not* apply at the cellular level.

Induction under "conditions of gratuity"

The cells are grown in an excess of lactate, a carbon source which does not interfere

References p. 393.

with induction. Enzyme synthesis is induced by the addition of β-methyl galactoside to the exponentially growing culture.

Induction here is not perfectly "gratuitous" since the enzyme, once formed, can hydrolyze the inducer, liberating galactose, which is utilizable. Ideally, a mutant unable to utilize galactose should be used, but this is not critical for these experiments.

In Fig. 7A, it is seen that, under these conditions, there is practically no time lag in the appearance of enzyme. The plot of enzyme formed against new growth (Fig. 7B) gives the typical straight line found by MONOD, PAPPENHEIMER AND COHEN-BAZIRE[11] under conditions of gratuity. The slope of the line corresponds to the specific activity of fully induced cells, signifying that, from the time of addition of the inducer, enzyme is synthesized in a fixed ratio to the synthesis of *new* bacterial protoplasm.

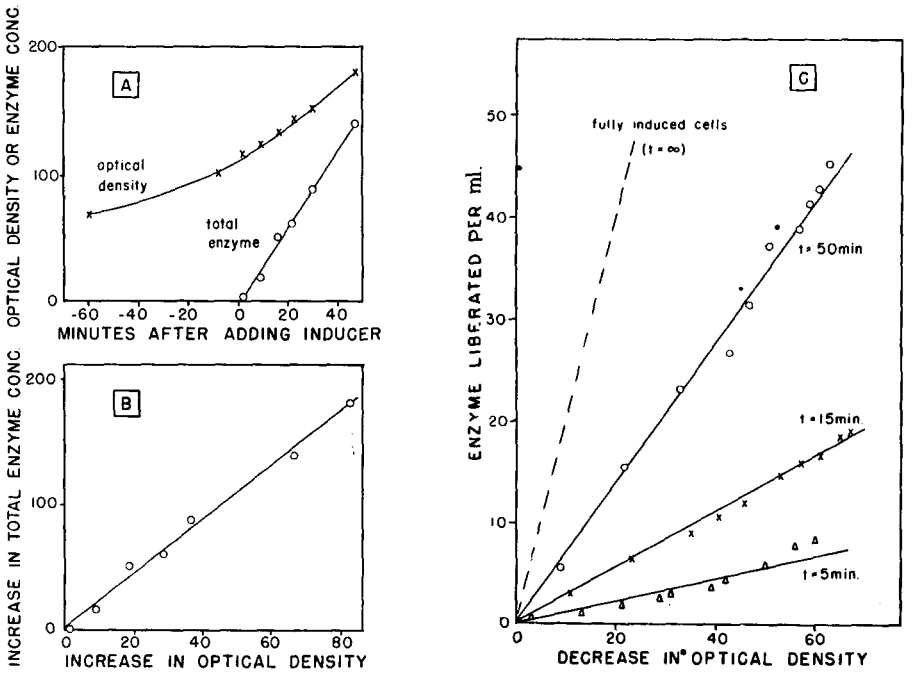

Fig. 7. Induction under "conditions of gratuity". Cells growing on lactate (0.2%) as non-inducing substrate are induced by the addition of β-methyl-D-galactoside at $t = 0$. A, B, and C as in Fig. 6. Enzyme concentration = units per ml.

As shown by the results of phage analysis (Fig. 7C), the population is essentially homogeneous in respect to enzyme content, *at all levels of specific activity*. Thus, all cells participate at comparable rates in the formation of enzyme, and the average kinetics gives a legitimate representation of the events in each cell.

DISTRIBUTION OF THE ENZYME AFTER DE-INDUCTION

In the system here studied, enzyme synthesis ceases when the inducer is removed. If enzymically active cells are allowed to multiply in the absence of inducer, their total enzyme content remains unchanged[15]. Thus "de-adaptation", in this case, simply

References p. 393.

amounts to a progressive dilution of the enzyme among the cells. By average observations on the culture, one cannot distinguish whether the enzyme initially present remains in a few cells or becomes uniformly distributed over all of them, but this question can be answered by the phage technique.

Cultures in various stages of enzyme "dilution" may be prepared by starting with fully active (lactose-grown) cells and allowing them to multiply on glucose as the non inducing substrate. Fig. 8A shows the optical density and enzyme as functions of time of growth on glucose; the degree of dilution at any time is simply the factor by which the culture has multiplied in the absence of inducer.

Fig. 8B shows the result of phage analysis of cultures diluted by factors of 8, 16 and 50 times. The straight lines indicate that the enzyme is uniformly partitioned among all the cells.

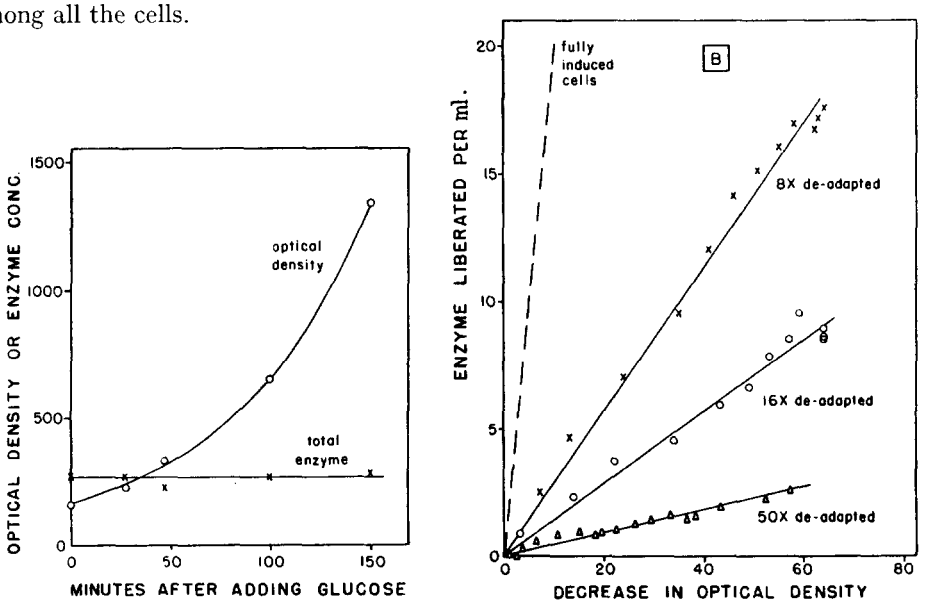

Fig. 8. Enzyme distribution after de-induction. Induced cells are prepared by growth on lactose. Twenty minutes after growth has ceased due to exhaustion of the lactose, glucose (0.2%) is added ($t = 0$).

A. Enzyme content per ml of culture and optical density as functions of time.

B. Analysis, by the phage procedure, of the cellular enzyme distribution in cultures of specific activity reduced to various degrees by growth on glucose.

DISCUSSION AND CONCLUSIONS

The induced synthesis of enzymes in bacteria, at least in the case studied, and under suitable conditions (gratuity), appears to be a process in which essentially all the cells participate to a similar degree. There is no inherent discreteness, the amount of enzyme in each cell rising gradually. Therefore the kinetics of the rise in overall specific activity of a population may be considered to represent substantially the events in the individual cells. This is definitely not true under *all* conditions, as shown by the experiments on induction by a substance that is also the only available carbon source. In this case, the individual cells produce enzyme at widely different rates.

The cellular uniformity of enzyme "dilution" after removal of the inducer implies:

References p. 393.

a. that the "new" and "old" protoplasm of a cell are equally distributed when division takes place; b. that the enzyme is dispersed within the cell. If the enzyme were associated with a number n of permanent and non-dissociating particles, the enzyme content of the cells should become markedly heterogeneous when the cells have multiplied by a factor n in the absence of inducer. The results in this case would suggest that n is greater than fifty.

This is still very far from the number of molecules of enzyme per fully active cell, which has been estimated at the order of 10^4 (MONOD AND COHN, personal communication) but it is difficult to extend this technique sufficiently to determine whether the intracellular enzyme is in the form of free molecules. It would be of interest to be able to test the homogeneity of 10^4 X diluted populations of cells, since one might be able to determine whether, indeed, every cell contains at least one molecule of the enzyme, as has sometimes been postulated.

Clearly, all the distributions measured by the phage lysis method appear more uniform than they really are, due to the limitations on resolution on account of lysis from without and physiological variability of the cells, so that a straight line result does not mean an absolutely uniform distribution. However, if as much as one third of the population contains twice the average amount of enzyme, an observable deviation from a straight line should be found. Conversely a *small* percentage of atypical cells would not be noticed. Since *optical density* is taken as a measure of *number of cells*, it is evident that what is measured is not strictly a *cellular* distribution, but one in which each "cell" is weighted according to its size.

The question of the number of independent *enzyme-forming* centers per cell is, as yet, untouched by these experiments. "Saturating" inducer concentrations have been employed, so that all such centers would be active. If the number of centers is small, then the use of low concentrations of inducer, or partial inhibition of enzyme synthesis (*e.g.* by the action of ultraviolet light) might lead to heterogeneous populations which could be detected by the phage technique.

The use of phage as an index to the state of an individual cell would appear to have general usefulness in a variety of problems in bacterial physiology where the proper interpretation of an overall observation depends upon the proverbial question of whether it represents a change in all or only some of the cells.

ACKNOWLEDGEMENTS

The author is deeply indebted to Dr A. LWOFF, Dr J. MONOD, and their collaborators at the Institut Pasteur for the privilege of *séjour* in the vital atmosphere of their laboratory, and for their assistance and teachings of varied character, too numerous to mention.

SUMMARY

Bacteriophage is utilized as a discriminating device for determining the distribution of an inducible enzyme (β-D-galactosidase) in the cells of a culture of bacteria (*E. coli* B). It is shown that the cellular distribution of enzyme is given by the shape of the curve of enzyme released *vs* number of cells lysed.

This distribution is investigated in cultures induced to various levels of specific enzyme activity. Under suitable conditions of induction, it is found that the induced synthesis of β-galactosidase proceeds at a uniform rate in all the cells. Under other conditions, however, a high degree of heterogeneity may occur.

When enzyme-containing cells multiply in the absence of inducer, the initially-present enzyme is uniformly partitioned among the daughter cells.

References p. 393.

RÉSUMÉ

La lyse par un bactériophage est utilisée comme méthode d'analyse de la distribution d'un enzyme inductible (la β-D-galactosidase) entre les cellules de cultures d'*Escherichia coli* B. Les expériences de contrôle démontrent que cette distribution peut être déduite de la forme de la relation entre enzyme libéré et nombre de cellules lysées.

La distribution de la β-galactosidase a été déterminée pour des cultures ayant atteint par induction différents niveaux d'activité enzymatique spécifique. Les résultats indiquent que lorsque les conditions sont adéquates, la synthèse induite de la β-galactosidase se produit avec la même vitesse chez toutes les cellules. D'autres conditions peuvent cependant créer un haut degré d'hétérogénéité.

Lorsque des cellules contenant l'enzyme se multiplient en l'absence d'inducteur, l'enzyme initialement présent est réparti uniformément parmi les cellules filles.

ZUSAMMENFASSUNG

Die Verteilung eines induzierbaren Enzyms (β-D-Galaktosidase) in den Zellen einer Bakterienkultur (*E. coli* B) wurde unter Verwendung von einem Bakteriophage als differenzierender Kunstgriff bestimmt. Es wurde gezeigt, dass die Verteilung des Enzyms in der Zelle gegeben ist durch die Gestalt der Kurve von freigesetztem Enzym gegenüber der Anzahl der lysierten Zellen.

Diese Verteilung wurde in Kulturen untersucht, die auf einen verschieden hohen Stand der spezifischen Enzymaktivität gebracht wurden. Unter passenden Induktionsbedingungen wurde gefunden, dass die induzierte Synthese der β-Galaktosidase mit einheitlicher Geschwindigkeit in allen Zellen verläuft. Unter anderen Bedingungen jedoch, kann ein hoher Grad an Ungleichheit vorkommen.

Vermehren sich die enzymenthaltenden Zellen bei Abwesenheit eines "Induktors", so wird das ursprünglich vorhandene Enzym gleichmässig auf die Tochterzellen verteilt.

REFERENCES

1 M. COHN AND J. MONOD, *Biochim. Biophys. Acta*, 7 (1951) 153.
2 M. DELBRÜCK, *J. Gen. Physiol.*, 23 (1940) 643.
3 N. H. HOROWITZ, *Advances in Genetics*, 3 (1950) 33.
4 F. JACOB, *Compt. rend.*, 232 (1951) 1780.
5 F. JACOB, *Ann. Inst. Pasteur*, 82 (1952) 578.
6 J. LEDERBERG, *J. Bact.*, 60 (1950) 381.
7 J. MONOD, A. M. TORRIANI AND J. GRIBETZ, *Compt. rend.*, 228 (1949) 718.
8 J. MONOD AND E. WOLLMAN, *Ann. Inst. Pasteur*, 73 (1947) 937.
9 J. MONOD, G. COHEN-BAZIRE AND M. COHN, *Biochim. Biophys. Acta*, 7 (1951) 585.
10 J. MONOD AND M. COHN, *Advances in Enzymol.*, 13 (1952) 67.
11 J. MONOD, A. M. PAPPENHEIMER AND G. COHEN-BAZIRE, *Biochim. Biophys. Acta*, 9 (1952) 648.
12 L. SIMINOVITCH AND F. JACOB, *Ann. Inst. Pasteur*, 83 (1952) 745.
13 S. SPIEGELMAN, in *The Enzymes*, SUMNER AND MYRBÄCK, eds., Vol. 1, part 1, p. 267. Academic Press, New York, 1951.
14 R. Y. STANIER, *Ann. Rev. Microbiol.*, 5 (1951) 35.
15 A. M. TORRIANI AND J. MONOD, Unpublished results.
16 C. B. FOWLER, *Biochim. Biophys. Acta*, 7 (1951) 563.

Received January 26th, 1953

APPENDIX

Ultraviolet resistance method

Another method of utilizing phage as a cellular testing device depends upon the fact that certain phages, during their intracellular reproduction, undergo progressive changes in resistance to inactivation by ultraviolet light (LURIA AND LATARJET, 1947; BENZER, 1952). By measuring the survival of plaque-forming ability *vs* dose for the population of infected bacteria, one can determine whether phage development proceeds uniformly or not in all the cells. If the culture conditions are properly chosen so that the factor in question (*e.g.* content of a particular enzyme) is rate limiting, the survival curves can give the distribution of this factor.

References p. 395.

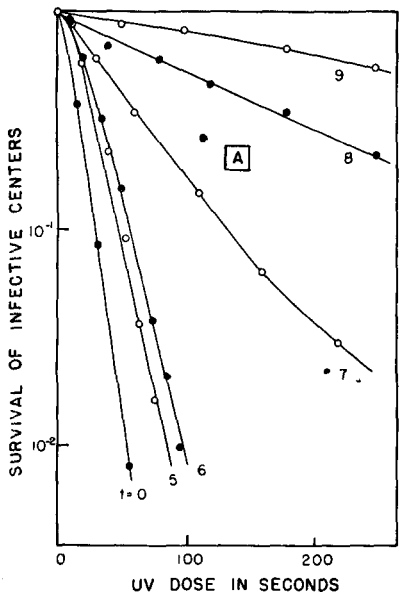

Fig. 9. Survival curves for T2r complexes irradiated after different times of development in broth. *E. coli* B taken from a culture growing exponentially in broth are washed, then incubated for one hour in buffer. Phage T2r (in buffer) is added and becomes absorbed to form complexes at an average multiplicity of $2 \cdot 10^{-3}$. Development is started by adding broth at $t = 0$. Temperature = 37° C.

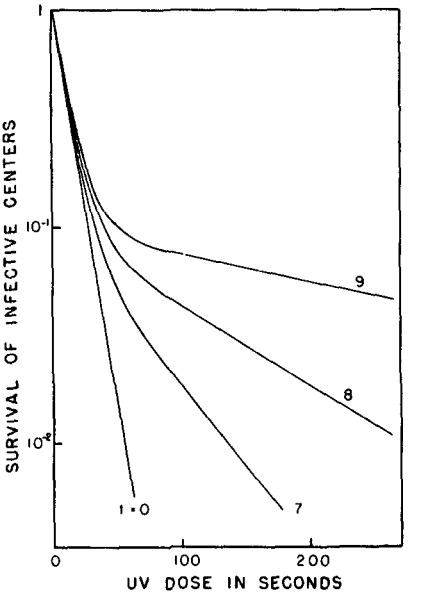

Fig. 10. Theoretical survival curves for a heterogeneous population of T2r complexes. It is assumed that 10% of the complexes develop at the full rate of Fig. 1, while 90% are unable to progress under the given conditions.

In Fig. 9 are shown experimental survival curves at different times for T2r-infected *E. coli* B, under conditions where all the cells can metabolize rapidly. In Fig. 10, by way of illustration, curves are drawn for a hypothetical heterogeneous mixture of cells, some of which can metabolize at the full rate, while others cannot at all. Bent curves such as these will not be obtained for a *uniform* population metabolizing at a diminished rate.

The following is an example of the application of the ultraviolet resistance method. We have seen, in the experiments on induction by a sole inducer-substrate, that some cells synthesize the enzyme sooner than others. This could be due to a variation in the amounts or availability of intracellular reserves (*e.g.* carbohydrate) in each cell. This conjecture can be tested by infecting the cells with phage T2r, in a medium complete except for a carbon source, and observing the survival curve as a function of time. In Fig. 11, the results are given for

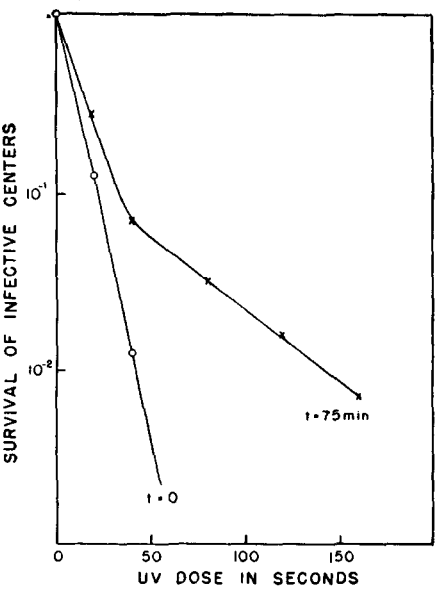

Fig. 11. Survival curves for T2r complexes formed from glucose-grown bacteria. *E. coli* B taken from a culture growing exponentially in synthetic medium plus glucose are washed and incubated for one hour in the absence of external carbon source. Phage T2r (in buffer) is added to form complexes at an average multiplicity of infection of $2 \cdot 10^{-3}$. These complexes are incubated *without adding any nutrients.*

References p. 395.

glucose-grown cells which have been washed and starved with respect to extracellular carbon. It is evident that phage develops to some extent even in these starved bacteria. The amount of development varies among the individual cells, presumably reflecting an uneven distribution of utilizable reserves. A similar experiment with broth grown bacteria shows no change in resistance beyond the value at $t = 0$.

The ultraviolet resistance measures a *cellular* (rather than an optical density) unit. It is not limited by lysis from without (only one phage particle per cell is necessary) and does not require that the factor studied be extractable from the cell. Therefore it may, in certain cases, have advantages over the lysis method used in this paper. The lysis method was preferred in this case because it was possible to demonstrate directly the activity of the enzyme released from the cells.

REFERENCES

S. BENZER, *J. Bact.*, 63 (1952) 59.
S. E. LURIA AND R. LATARJET, *J. Bact.*, 53 (1947) 149.