ANALYSIS OF THE DIFFERENTIATION AND OF THE HETEROGENEITY WITHIN A POPULATION OF *ESCHERICHIA COLI* UNDERGOING INDUCED β-GALACTOSIDASE SYNTHESIS[1]

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The kinetics of induction of the galactozymase of certain yeasts (Spiegelman, 1951) and the permease-β-galactosidase system of *Escherichia coli* (Monod, 1956) can, under certain conditions, be described as autocatalytic. Essentially two hypotheses have been proposed to account for such findings: (a) that the enzyme-forming system itself is autocatalytically activated (Campbell and Spiegelman, 1956) or self-reproducing (Spiegelman, 1946), or (b) that in some way the enzyme being induced intervenes in its own induction (Monod and Cohn, 1952).

To evaluate these hypotheses, it should be recalled that the kinetics of formation of an enzyme by a population of cells describes events at the cellular level only if the response of each individual cell is simultaneous and equal (Benzer, 1953; Monod and Cohn, 1952). Such a response we term homogeneity. The first experimental analysis of this problem we owe to Benzer (1953) who defined the experimental conditions under which the induction of β-galactosidase would proceed in a homogeneous fashion in an *E. coli* (lac^+) population. These were (a) conditions of gratuity, i. e., neither the presence of the enzyme itself nor its inducer influences general cellular metabolism, and (b) a saturating concentration of inducer. Benzer (1953) also showed that heterogeneity is generated under conditions of nongratuity. The question remained however as to what the response would be at nonsaturating concentrations of inducer.

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Novick and Weiner (1957, 1959) and Novick and McCoy (1958) took up this problem and were able to demonstrate that under conditions of gratuity, at nonsaturating concentrations of inducer, the response of the *E. coli* population was heterogeneous. The most remarkable point about these findings was that at a given low inducer concentration (a) all the individuals of the population were in either one of the two possible states, maximally induced or noninduced. because the transition state from noninduced to induced is of very short duration, and (b) the induced state was clonally distributed. Also, the descendants of the noninduced cells were in general noninduced. However, with a given frequency, depending on inducer concentration, the noninduced cell was converted to an induced one.

That the induction of an enzyme system could be clonally distributed was first found with long-term adapting yeasts which were discovered by Winge and Roberts (1948) and extensively investigated by Spiegelman (1946, 1951, 1954; Spiegelman and DeLorenzo, 1952; Spiegelman *et al.*, 1951, 1950). The induced formation of the galactozymase system of these yeasts was not only clonally distributed but also cytoplasmically inherited. In interpreting this finding, Spiegelman (Campbell and Spiegelman, 1956; Spiegelman, 1951, 1954; Spiegelman and DeLorenzo, 1952; Spiegelman *et al.*, 1951, 1950) proposed that the clonal distribution was due to self-reproduction or autocatalytic activation of the site of formation of enzyme template.

As pointed out at the start, an alternative model which did not assume any entities other than enzymes and inducers also accounted for the distribution (Monod and Cohn, 1952) of induced cells found in the case of the long-term adapting yeasts.

The model supposed that in a suspension of growing cells, an inducer, I, provokes the synthesis of an enzyme, Y. Furthermore, for reasons

to be discussed later, the probability that any cell will synthesize at least 1 molecule of Y per unit time is taken to be: (a) infinitesimal in the absence of I; (b) small in the presence of I for those cells which do not possess already one molecule of Y; and (c) large in the presence of I for the cells which possess already one molecule of Y.

In such a system the synthesis of enzyme will be a phenomenon practically discontinuous at the cellular level by comparison with the over-all kinetics. If the probability of formation of the first molecule of Y within a generation time is small, then the discontinuity in question would not only be reflected in a given cell but during a certain time in the descendants. At the cellular level the capacity to rapidly synthesize the ensyme, Y, would become a clonal property appearing like a mutation, while at the population level, the adaptation in the presence of inducer would be slow and would extend over a rather large number of cellular generations.

From what has been already established in the *E. coli* system (Cohn, 1957; Novick and Weiner, 1957), if I is equated to internal inducer and Y to galactoside-permease, then this model predicts both the maintenance effect (Cohn and Horibata, 1959; Novick and Weiner, 1957) and the clonal distributions of induced cells (Novick and Weiner, 1957).

The above model, when it was originally proposed, did not specify why the probability of a cell being induced would be increased by the presence of Y. The clonally distributed heterogeneity would simply arise whenever one or a few molecules of Y greatly increased the probability of induction. In the case of the galactoside-permease it is evident this should be so since it is induced by its own product, internal inducer, and not by its substrate, external inducer.

In preceding papers it was shown that a preinduced cell (lac^+) placed into glucose and inducer (Cohn and Horibata, 1959) or at low inducer concentration (Novick and Weiner, 1957) would continue to make galactoside-permease and β-galactosidase indefinitely, whereas the noninduced cell under identical conditions would not synthesize either permease or β-galactosidase. These alternate steady states (maintenance effect) were proved to be linked to the presence or absence of galactoside-permease which is responsible for the uptake and concentration of inducer in the cell (Rickenberg *et al.*, 1956). The maintenance effect then could be and was used by Novick and Weiner (1957) as a tool to analyze the state of induction of the individuals in a population. The goal of this paper is to extend the evidence of Novick and Weiner (1957) that the clonal distribution of the response of the population to induction under certain conditions is due to the properties of the permease by actually carrying out direct measurements of permease activity. Furthermore, the findings with the *E. coli* system will be discussed with relation to those in the yeast system to see to what extent each of the two above discussed hypotheses are tenable.

MATERIALS AND METHODS

The techniques used for the cultivation of the bacteria, the assays of enzymatic activity and induction, and the various mutants used have been described in the previous paper (Cohn and Horibata, 1959). The only difference has been in the assay of galactoside-permease which was carried out using radioactive S^{35}-labeled β-D-thiogalactosido β-D-galactoside (TDG) and instead of separating the bacteria by centrifugation, filtration on Millipore membranes was used (Cohn *et al.*, 1959).

The pertinent properties of TDG[4] have been discussed by Cohen and Monod (1957) who showed that it had a higher affinity for the galactoside-permease than methyl β-D-thiogalactoside (TMG) and that it was, at best, a poor inducer of the β-galactosidase system.

EXPERIMENTAL RESULTS

Assay for homogeneity of the population. The previous paper (Cohn and Horibata, 1959) showed that preinduced ML30 (lac^+) cell when placed in a mixture of glucose and TMG would produce both β-galactosidase (Z) and galactoside-permease (Y), whereas a noninduced cell under identical conditions would synthesize neither. This fact provides an excellent tool for analyzing the state of induction of the population. The validity of this analysis is confirmed by the reconstruction experiment shown in figure 1. A fully induced population of ML30 was mixed in varying proportions with a noninduced culture

[4] We wish to express our indebtedness to Dr. Dietmar Türk for having made this compound.

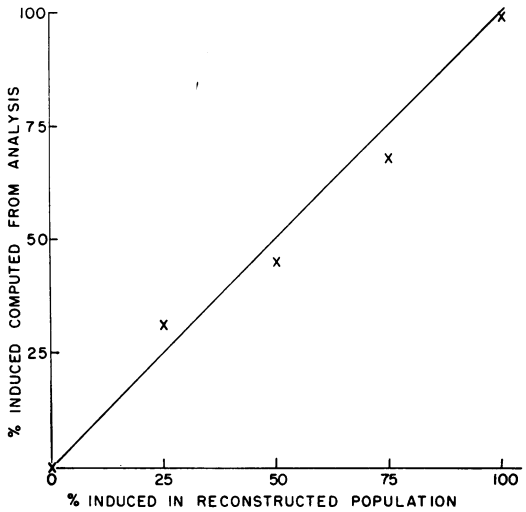

Figure 1. Reconstruction experiment to test analysis procedure. A fully induced culture of ML30 and a noninduced culture were mixed in varying proportions and diluted to an average level of 0.6 to 1.0 bacterium per tube into 200 tubes containing succinate 56 medium with glucose, 10^{-3} M, and methyl β-D-thiogalactoside (TMG), 10^{-4} M. The tubes were incubated aerobically at 37 C. When the concentration of cells reached 10^7 to 10^8 cells per ml, the optical density was determined and the culture was treated with toluene for assay of enzyme activity. A positive is scored as one containing a specific activity of more than 20 units per μg N. A negative contained less than 0.2 units per μg N. There were none between these values. No growth in 48 hr was considered as indicating a tube which had been given no bacteria. Corrections were applied for tubes containing 2 or more bacteria at the start. This will be illustrated in table 2. The line is the theoretically expected result.

and the mixture was analyzed using the maintenance effect as a tool.[5]

It is clear that this analysis is in close agreement with expected values. Novick and Weiner (1957) have used maintenance on low concentrations of TMG to analyze populations undergoing induction. The two methods of analysis by maintenance in glucose and at low inducer concentration should be equivalent if the selfinduction hypothesis described previously (Cohn and Horibata, 1959) is valid.

Application of the assay for heterogeneity to cultures undergoing induction. It is known that the constant differential rate of the induced synthesis of β-galactosidase is found under two circumstances: (a) when saturating concentrations of certain alkyl-galactoside inducers are used with strains which are inducible for both galactoside-permease (Y) and β-galactosidase (Z) (Cohn, 1957; Monod, 1956; Novick and Weiner, 1957), and (b) when any effective concentration of inducer is used with strains which cannot form the Y system but can make Z (Herzenberg, 1958). These findings of Monod (1956) and Herzenberg (1958) are confirmed by the experiment illustrated in figure 2. With ML30 (Y^+Z^+), at concentrations of inducer above 10^{-4} M, there is a constant differential rate of synthesis within 5 min of addition of inducer. As the inducer concentration is decreased, an acceleration phase in the rate of enzyme formation is observed. At 4×10^{-5} M TMG, the differential rate of synthesis reaches a constant maximum after 2 to 3 divisions, whereas at 10^{-5} M TMG it does not reach a constant value even after many divisions (figure 2).

On the other hand, ML3, the lac^- cryptic (Y^-Z^+), shows a constant differential rate from the time of addition of inducer no matter what its concentration (Herzenberg, 1958).

This difference in behavior between ML30 (Y^+Z^+) and ML3 (Y^-Z^+) is linked to the presence of the galactoside-permease, Y (Herzenberg, 1958). Since the permease possesses the essential properties ascribed to Y in the model proposed in the introduction, then its presence should lead to a heterogeneity of the population. The maintenance effect allows this point to be tested.

Consider the induction of ML30 (Y^+Z^+) (figure 2) by saturating concentrations of TMG ($> 10^{-4}$ M). At time zero the noninduced culture when analyzed shows no preinduced cells, as would be expected (table 1). Fifteen minutes later, however, the entire population is induced. This finding that at saturating concentrations of inducer the response of the population is homogeneous,[6] is a confirmation of the work of Benzer

[5] There is the implicit assumption that a fully induced cell from a culture grown in high inducer concentration is equivalent to a fully induced cell from low inducer concentration.

[6] Time is implicitly involved in the distinction between homogeneous and heterogeneous. What is really measured is the time for the population to become homogeneous. Experimentally, a homoge-

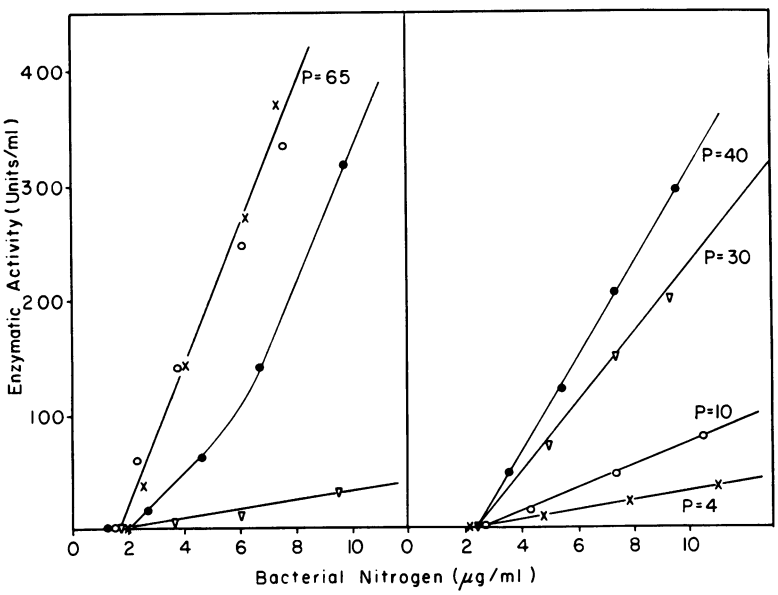

Figure 2. The induction of β-galactosidase by varying concentration of methyl β-D-thiogalactoside (TMG) in *Escherichia coli* strain ML30 (Y^+Z^+) (*left*) and in *E. coli* strain ML3 (Y^-Z^+) (*right*). Induction of strain ML30 was carried out at the following concentrations of TMG: 1 x 10^{-3} M (○——○), 1 x 10^{-4} M(×——×), 4 x 10^{-5} M (●——●), and 1 x 10^{-5} M (▽——▽).

Induction of strain ML3 was carried out at TMG concentrations: 1 x 10^{-2} M (●——●), 1 x 10^{-3} M (▽——▽), 3 x 10^{-4} M (○——○), and 5 x 10^{-5} M (×——×). P is the differential rate of synthesis (Benzer, 1953; Cohn and Horibata, 1959; Monod *et al.*, 1952).

(1953) by another method. However as the inducer concentration is lowered, the population responds in a heterogeneous[6] fashion. At 4 × 10^{-5} M TMG, after 60 min, the population consists of 9 per cent preinduced and 91 per cent noninduced. At 180 min the culture is homogeneous. At 10^{-5} M TMG even after 240 min only 21 per cent of the cells are producing the β-galactoside system (Y and Z). This experiment confirms then the findings of Novick and Weiner (1957).

Unfortunately the maintenance technique cannot be applied to permease-less organisms, e.g., ML3 (Y^-Z^+).[7] There are, however, two arguments which suggest that the constant differential rate of synthesis shown by ML3 at all inducer concentrations, results from a homogeneous response to induction: (a) With the ML30 (Y^+Z^+) strain the constant differential rate of synthesis has been shown experimentally to be concomitant with a homogeneous response (table 1) and this finding might be extrapolated to ML3 (Y^-Z^+), and (b) there is no likely distribution of properties which would account for a constant differential rate of induction.

The argument which has been presented implies, of course, that the primary event in the development of discontinuity in the population is at the level of Y and not Z which simply mirrors the state of induction of Y. This has been shown (table 2) in a rather qualitative way by carrying out at low inducer concentration the induction of Y in strain W2242 (Y^+Z^-) which lacks β-galactosidase. The culture undergoing induction was diluted to the level of about one organism per tube into maintenance concentrations of glucose and TMG as described previously (Cohn and Horibata, 1959). The resultant clones were centrifuged and resuspended in medium with radioactive TDG (5 × 10^{-4} M).

Although there was a wide spread in the Y activity (0.1 to 0.5 mμmole/μg N), there was a sharp break below 0.1 where only basal activities

neous response is one which is attained within 10 min of addition of inducer. See Cohn (1957) for further discussion.

[7] The Benzer (1953) technique is applicable but has not yet been attempted.

were found (approximately 0.02). Negatives were defined as those clones with below 0.1 units Y activity. It is clear that at saturating concentrations (culture 1, table 2) the induction is virtually homogeneous, 93 per cent being positive whereas at low concentrations there are a small proportion of positives, 8.7 per cent which increases with time 15.8 per cent. This experiment shows that a heterogeneous response is possible during the induction of Y alone.

Dilution of the Y system during growth in the absence of induction. (1) Conditions of stability of Y:—It should be recalled that the β-galactosidase itself is stable in the cell so that during growth of the culture in the absence of inducer, the enzyme level per ml of culture remains constant and the specific activity falls as a linear function of growth (Benzer, 1953; Monod and Cohn, 1952; Novick and Weiner, 1959). Under conditions where Z is stable, Y is not (figure 3).

First of all, repeated washing to eliminate inducer causes loss of Y activity (compare starting activities of curves 1, 2, 3 and curves 4, 5, 6). This washing by inactivation was not prevented simply by adding reducing agents to the medium (curve 3).

TABLE 1

Analysis of the state of homogeneity of the population of strain ML30 undergoing induction

Conc Methyl β-D-thiogalactoside (TMG)	Time after Start of Induction (min)					
	0	15	60	120	180	240
M						
10^{-3}	<2	>96	>96	—	—	—
10^{-4}	<2	>96	>96	—	—	—
4×10^{-5}	<2	<2	9	33	>96	>96
10^{-5}	<2	—	2	8	15	21

The noninduced culture of ML30 used in the experiment described in figure 2 was assayed for its composition in induced and noninduced cells as defined in figure 1. Two simplifications were introduced. Growth of the clones was stopped by toluene when the concentration was between 10^7 to 10^8 cells per ml as judged visually. *o*-Nitrophenyl β-D-galactoside was added and the cultures which turned yellow within 10 min were scored positive. The negatives showed nothing in 60 min. Experience has shown this rough technique to be sufficient for most analyses.

The figures are the percentage of positives in the population.

TABLE 2

Response to induction of Y in W2242 (Y^+ Z^-)

Designation	Time (1)	Y Activity (2)	Total No. of Tubes (3)	Tubes with No Growth (4)	Positive (5)
	min	*mμmole/μg N*		%	%
Mother culture.	0	<0.02	30	63.4	0
Culture I, 5×10^{-4} M TMG..	60	0.13	30	53.4	93
Culture II, 3×10^{-5} M TMG..	60	0.03	50	54.0	8.7
	130	0.06	50	62.0	15.8

A noninduced culture growing on succinate was divided into two parts, I and II, containing a saturating and nonsaturating concentration of methyl β-D-thiogalactoside (TMG). The growth rate of the cultures was 0.86 div/hr. The starting concentration of bacteria was 5 μg N per ml.

At the times indicated the cultures were assayed for permease activity using β-D-thiogalactosido β-D-galactoside (TDG) and diluted as discussed in figure 1 into maintenance conditions: glucose 10^{-3} M and TMG 5×10^{-4} M when the bacterial density was about 5 μg N/ml as measured by optical density, permease activity was assayed with TDG (see text). An activity of >0.1 mμmole per μg N was scored as positive. The negatives ranged around 0.02, the limit of measurement. A fully induced control grown in the absence of glucose showed Y activities of 0.3 to 0.4 mμmole per μg N.

Therefore, attempts were made to stop induction by the use of the noninducing TMG analogues, β-D-thiogalactosido β-D-galactoside (TDG) and phenyl β-D-thiogalactoside (TPG). The addition of TDG did not completely block induction (curve 4), there being a significant but small continued synthesis of Y. TPG effectively blocks Y synthesis and under the conditions described by curve 5 there is dilution of the Y system. However, there is a significant destruction of Y activity (approximately 20 per cent per division) not clearly shown under the conditions of this experiment, where high cell densities were used in order to measure Y. At low cell densities, necessary for the experiment to be described next, this destruction had to be avoided. On the assumption that Y inactivation is time dependent and not growth dependent, nutrient broth was added to the medium to increase the growth rate 2-fold and make a dilution experi-

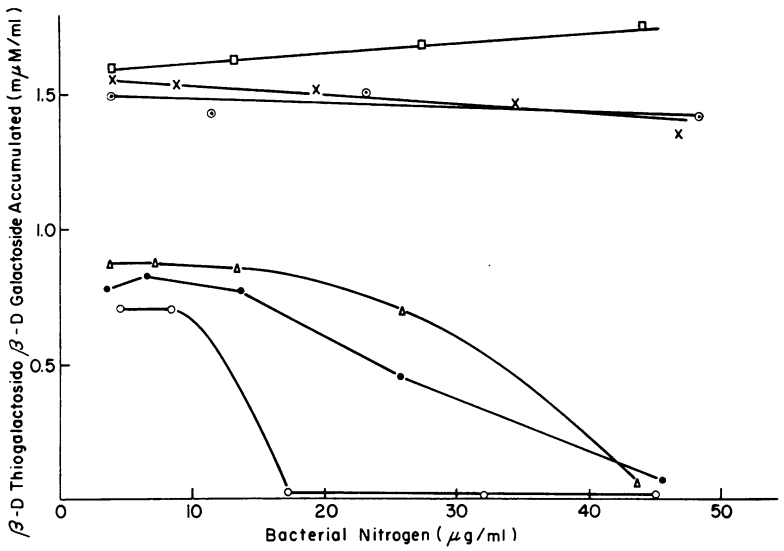

Figure 3. Dilution of the Y system upon growth in the absence of inducer under different conditions. A preinduced culture (ML30) growing in succinate 56 medium with (TMG) (10^{-4} M) was treated as follows: ●——● = Curve 1, washed 5× with cold succinate 56 medium to eliminate inducer and reinoculated into succinate 56 medium to grow; ○——○ = curve 2, washed 3× as above with NaN_3 (10^{-2} M) added to wash solution. Reinoculated as above; △——△ = curve 3, washed 5× as above but 2-mercaptoethanol (10^{-3} M) was added to both wash and growth medium. In addition, glucose (10^{-2} M) added to growth medium; □——□ = curve 4, preinduced culture without washing diluted 2-fold into growth medium containing β-D-thiogalactosido β-D-galactoside (TDG) in a concentration such that finally TDG was 2×10^{-3} M and TMG, 5×10^{-5} M; ×——× = curve 5, preinduced culture without washing was diluted 2-fold into a growth medium containing phenyl β-D-thiogalactoside (TPG), 2-mercaptoethanol, and glucose such that the final concentrations were TPG, 2×10^{-3} M; 2-mercaptoethanol, 5×10^{-4} M; TMG, 5×10^{-5} M; and glucose, 10^{-2} M; and ⊙——⊙ = curve 6, same as 5 except that nutrient broth (Difco) 1 per cent was added to medium.

In assaying for Y, the aliquots were rapidly centrifuged and resuspended in the test medium to avoid any possible complications due to the various constituents added to growth media.

ment possible in much less time. In the presence then of nutrient broth and other constituents (curve 6) the Y activity per ml remained constant and like the enzyme itself was simply diluted in the growing population.[8]

(2) Production of heterogeneity:—The following model had been derived from the analysis of Spiegelman *et al.* (1951). A preinduced cell contains Yo units of permease of which one is sufficient to bestow maintenance. These units are distributed at random to the daughter cells and are neither synthesized nor destroyed during growth in the absence of induction. If

Po = fraction of cells with 0 units, (negatives)
Yo = average number of units per cell at the start, (n = 0)
n = number of generations starting with 0 generations
then $Yo\ 2^{-n}$ = average number of particles per cell at the n^{th} generation
and Po = $e - Yo\ 2^{-n}$.

Converting to logarithms: $\log_2 \ln 1/Po = (\log_2 Yo) - n$. The plot of $\log_2 \ln 1/Po$ *versus* n should give a line of slope −1 extrapolating to give $\log_2 Yo$ at n = 0 from which the value of Yo is obtained.

The experiment based on this model was carried out by allowing the preinduced culture to dilute out its Y system in the absence of induc-

[8] The assumption is that we do not have a situation in which synthesis of Y equals destruction of Y.

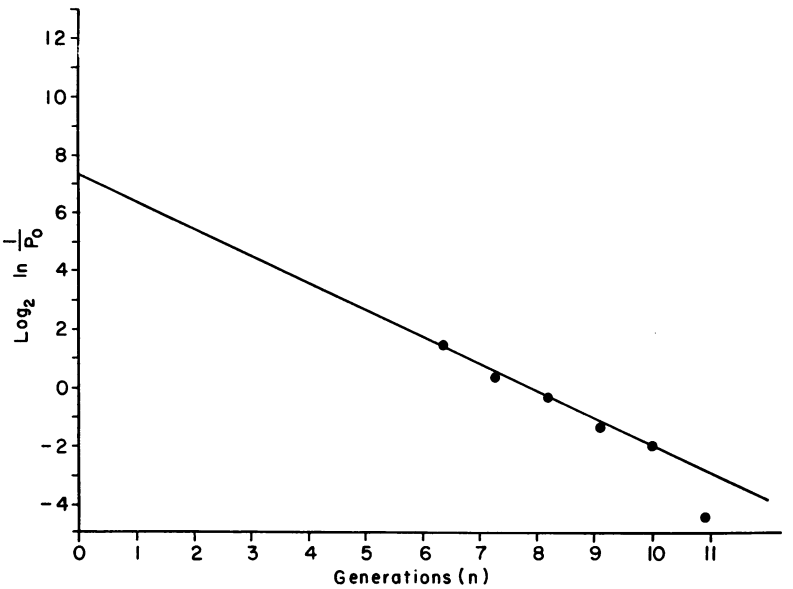

Figure 4. The kinetics of the dilution of the maintenance system during growth in the absence of induction. See table 3 for data.

TABLE 3

Appearance of heterogeneity in a preinduced population of Escherichia coli strain ML30 during growth in the absence of induction

Time	Generations (n)	Total No. Tubes Inoculated	No. Tubes Showing Growth	No. Tubes Showing No Growth	Negatives	Proportion Negatives (Po)	Avg Bacteria/ Tube (m)	Po Corrected for Over 2 Bacteria/ Tube	$\ln \frac{1}{Po}$	$\text{Log}_2 \ln \frac{1}{Po}$
hr										
2.0	3.64	30	10	20	0	—	—	—	—	—
2.5	4.54	30	15	15	0	—	—	—	—	—
3.0	5.45	100	48	52	0	—	—	—	—	—
3.5	6.36	100	45	55	3	0.0667	0.60	0.088	2.42	1.37
4.0	7.27	100	59	41	13	0.221	0.89	0.29	1.24	0.310
4.5	8.18	100	35	65	15	0.429	0.43	0.46	0.782	−0.355
5.0	9.09	100	62	38	34	0.549	0.97	0.68	0.386	−1.37
5.5	10.0	100	41	59	32	0.780	0.53	0.78	0.245	−2.03
6.0	10.9	100	52	48	50	0.962	0.73	0.96	0.0398	−4.65

A preinduced culture was allowed to dilute out its Y system under conditions described by curve 6, figure 3. At the times indicated the culture was assayed for the number of preinduced cells as described in figure 1.

The generation time of the culture was 33 min at 34 C. The starting concentration of bacteria was 3×10^5 per ml.

Criteria for negatives and no growth are in the legend to table 1 and figure 1.

Sample calculation for 3½-hr sample:

Proportion negatives (Po) ..... = 3/45 = 0.0667

Proportion of tubes showing no growth ..... = 55/100 = 0.55 = $_e-m$

Average bacteria per tube ..... = m = 0.60

Proportion negatives corrected for 2 or more bacteria per tube the sum of terms of Poisson distribution for 2 and above ..... = 0.12

$2 \times 0.12 \times 0.0667 \times 0.933 \times 45 = 0.67$ positive or 1 positive

3 + 1 = 4 positives 4/45 = 0.088 the value of Po corrected for over 2 bacteria per tube.

tion under the conditions of curve 6, figure 3. After six divisions, negatives were found in the population and the proportion of negatives which appeared follows the model just described (figure 4, table 3). However, the actual question is not with which model these data are compatible, but with which model they are incompatible. The error in these data is such that they are incompatible with 3 or more permease units being necessary to confer positivity but still compatible with 1 or 2 units.[9] The importance of these data is that they show that very few units of permease, about 200 ± 100, are present in the fully induced cell and of these only 1 or 2 are needed to confer mantenance on a cell under the specified conditions.

It should be stressed that the above experiments are describing permease units as defined by the distribution data. One unit is not necessarily 1 *molecule* of permease. The unit could consist of a particle with many molecules of permease, analogous to the particles of yeast carrying many cytochrome molecules described by Ephrussi (1953) and Slonimski (1952). However, since 100 to 200 Y units are present in the cell which has with the best substrate (TDG), a maximum activity at 37 C of 0.2 mμmole per min per μg N (Cohen and Monod, 1957), then the maximum turnover number of Y is 1000 molecules per sec per unit Y. This value is not high for a simple enzyme reaction and is, therefore, compatible with the hypothesis that 1 unit of Y is actually 1 molecule.

The above described data agree quite well with the recent findings of Novick and McCoy (1958) and Novick and Weiner (1959) who have used the kinetics of the loss of maintainability of a preinduced population growing in the absence of inducer to estimate (a) the total number of permease units per induced cell and (b) the minimum number which can confer maintenance. This method is capable of much greater accuracy in estimating the number of permease units than the distribution experiment described here. If a more accurate value of Po were needed, kinetic analysis should be repeated under the present conditions where no destruction of Y is detectable.

[9] This was done as described by Spiegelman (1951) by means of curve fitting.

DISCUSSION

The goal of this study has been to see whether the model (Monod and Cohn, 1952) originally proposed to account for the findings with a long-term adapting yeast (Campbell and Spiegelman, 1956; Spiegelman, 1951, 1954; Spiegelman and DeLorenzo, 1952; Spiegelman *et al.*, 1951, 1950; Winge and Roberts, 1948), could be applied to the β-galactosidase system. In any induced system whenever the further formation of a given enzyme is sharply dependent upon its own activity, then discontinuities can arise in the synthesis of this enzyme by individual cells. This is a restatement of the concept of gratuity (Cohn, 1957; Monod and Cohn, 1952) according to which the kinetics of formation of an induced enzyme by a bacterial population is most simply studied under conditions where the enzyme itself is neither a necessary link in the general intermediate metabolism of the cell nor is in the pathway by which the inducer reaches its site of action in the cell. The studies with the galactoside-permease illustrate these points. When lactose is used as only carbon and energy source as well as inducer of the β-galactosidase system, the induction of the system is heterogeneous in the population, as Benzer (1953) demonstrated. Since both the galactoside-permease and the β-galactosidase itself are essential links in the conversion of lactose to metabolites and since the permease alone is critical in the pathway of induction by lactose, it is not surprising that cells which possess a trace of Y have a tremendous advantage in being further induced over those which have none. This advantage at first was eliminated by simply providing the culture with an independent energy source and a saturating concentration of a nonmetabolizable inducer such as an alkyl β-D-thiogalactoside. Under these conditions the response of the population is homogeneous (Benzer, 1953). However, as the external concentration of inducer is lowered, the generation of an effective concentration of inducer at its site of action in the cell becomes increasingly dependent upon the Y system which in turn is being induced. Cells with a trace of Y then have a high probability of being further induced, whereas those with no Y are not induced and heterogeneity appears in the population. Alkyl-galactosides can enter the cell via a pathway, e. g., diffusion, which does not involve Y. This is

illustrated by the cryptic strain ML3 (Y^-Z^+) which is inducible for β-galactosidase at external concentrations of alkyl-galactosides higher than those required for ML30 (Y^+Z^+). At saturating concentrations of inducer, the Y system does not give any advantage to a cell which has some Y and the response to induction appears to be uniform in the population. In ML30, the Y^+Z^+ strain, β-galactosidase reflects the state of induction of Y and heterogeneity in the synthesis of β-galactosidase implies heterogeneity in the synthesis of Y. The implication is supported by the demonstration that W2242, the Y^+Z^- strain, responds at low inducer concentration in a heterogeneous fashion with respect to Y synthesis alone.

The phenomenon described here is reminiscent of long-term adaptation to galactose fermentation by yeast (Spiegelman, 1951, 1954; Spiegelman and DeLorenzo, 1952; Spiegelman *et al.*, 1951, 1950). Certain strains of yeast (fast fermenters) when placed on a growth medium containing galactose as essential carbon and energy source, grow on and ferment this sugar after a short lag. Other strains (slow fermenters) do so only after a prolonged lag. If one plates the adapting slow fermenting population onto a suitable solid medium containing galactose, the resultant colonies or clones are of two kinds: negatives which do not ferment or grow on galactose and positives which do. The response of this population to galactose is, therefore, a heterogeneous one. The ability to record as positive on a solid medium depends on the continued presence of galactose (maintenance) and growth in the absence of galactose leads to a reversion of the population to the negative phenotype. The factor conferring positivity is not segregated in a Mendelian fashion and is therefore defined as cytoplasmic (Spiegelman and DeLorenzo, 1952).

One model which had been proposed (Campbell and Spiegelman, 1956) to explain the above phenomenon identified the cytoplasmic entity conferring positivity with one of the active enzyme-forming systems (templates) involved in galactozymase synthesis. It was stated that "all the data can be described in a unified fashion by the assumption that initially the templates are relatively inactive and are autocatalytically converted to full activity during the course of induction" (Campbell and Spiegelman, 1956).

This hypothesis will be termed the "template model." The hypothesis used here to describe the *E. coli* system will be called the "self-induction model."

Can the yeast system and *E. coli* system be explained in terms of a single hypothesis? First, how can the *E. coli* system be understood if the template model is invoked? The evidence presented here is that the induced phenotypic variation with regard to β-galactosidase synthesis results from the presence of the galactoside-permease. It is clear that the Y system does not form part of the enzyme-forming system (template) for the β-galactosidase synthesis since mutants which lack Y, make Z quite as well as those which have Y. Therefore the template model is inapplicable to the *E. coli* system.

Is the self-induction model applicable to the yeast system? The utilization of galactose involves several enzymes. The existence of a permeation system is suggested by the work of Douglas and Condie (1954). After entry into the cell, galactose is phosphorylated by the action of a kinase and then converted to glucose 1-phosphate in two enzymatic steps (Kalckar *et al.* 1958). The entire enzyme system involved in the complete fermentation of galactose is known as galactozymase, of which the first two or three steps are inducible. If the product of the first reaction were a good inducer of the second enzyme, then a trace of the first enzyme would greatly favor the induction of the second. Such a trace could be below detectability by any available assay methods for galactozymase activity either by direct or indirect measurement using growth on galactose (Campbell and Spiegelman, 1953). No experiment so far published has ruled out the hypothesis that the cytoplasmic particle, above postulated, is, in reality, one of the induced enzymes (possibly the permease) involved in the galactozymase pathway. All that is needed to apply the selfinduction model is that one of the enzymes of galactozymase behave as Y in the model.

Furthermore, the genetic studies of Spiegelman (Spiegelman and DeLorenzo, 1952) imply that there is a cytoplasmically inherited particle which confers on a cell the ability to grow rapidly on galactose. Clearly one of the enzymes of the galactozymase system would be expected to behave as just such a particle and there is there-

fore no contradiction between the genetic findings and the self-induction hypothesis.

The question is raised then as to what the difference might be between fast and slow fermenters according to the self-induction model. There are several possibilities. For example, slow strains could require higher internal concentrations of inducer than fast strains to produce enzyme or fast strains could possess the critical enzyme constitutively whereas the slow strains do not. Therefore we must conclude that there is no direct evidence for the self-reproducing autocatalytically activated template proposed (Campbell and Spiegelman, 1956) to account for the findings with long-term adapting yeasts. The self-induction model is preferable because (a) it is generally applicable (both to *E. coli* and yeast) and (b) it is simple, requiring no assumptions concerning templates or self-reproducing particles since this model is based only on measurable and chemically defined entities, enzymes, permeases, and inducers.

It might be valuable to end this discussion with a comment about the all-or-none nature of enzyme induction at the cellular level, a concept which arises from the studies of Novick and Weiner (1957). The model is that when a bacterium possesses its first permease unit, "the internal inducer concentration is raised, and the probability of the appearance of a second permease unit is sharply increased. In this sense the induction of the 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 a maximum rate. Because the transition is accomplished so rapidly, the relative number of bacteria at an intermediate state of induction is small" (Novick and Weiner, 1957). The all-or-none property of the induction of the permease-galactosidase system is defined then by the clonal distribution of induced bacteria as revealed by the use of maintenance conditions. One cannot extrapolate from the above described all or none behavior of a bacterium to the level of the enzyme-forming system by concluding that saturation by inducer is obligatory to enzyme synthesis which would show no intermediate rates. It is already known that both the cryptic ML3 (Y^-Z^+) strain ((Herzenberg, 1958 and (figure 2)) and the preinduced ML30 (Y^+Z^+) strain (Monod, 1956) show intermediate levels of induction as the inducer concentration in the growth medium is lowered. Therefore, the all-or-none distribution of induction cannot as yet be interpreted to reflect a property of the enzyme-forming system itself.

SUMMARY

Under conditions where the metabolic activity of a system under induction is essential to the formation of that system, then the response of each cell in a population is sharply dependent upon whether it possesses a minimum unit of the induced activity. As a result, the formation of enzyme by the population is heterogeneous. Both the formation and nonformation of enzyme is passed on clonally to the descendants. Two induced systems: the galactoside-permease and β-galactosidase (Cohen and Monod, 1957) of *Escherichia coli* and the galactozymase of long-term adapting yeast (Spiegelman, 1951) can be understood in terms of this concept.

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