PHYSIOLOGY OF THE INHIBITION BY GLUCOSE OF THE INDUCED SYNTHESIS OF THE β-GALACTOSIDE-ENZYME SYSTEM OF *ESCHERICHIA COLI*[1]

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In previous papers a model (Cohn, 1956, 1958; Cohn and Horibata, 1959*a*, *b*) was described which completely rationalized the kinetics of the induction by galactosides and the inhibition by glucose in terms of the properties of the galactoside-permease. However, little information was provided on the mechanism by which glucose acts as an inhibitor of induction. It is known (Cohn and Horibata, 1959*a*, *b*; Herzenberg, 1958) that glucose inhibits induced β-galactosidase synthesis in mutants which lack permease, and, conversely, induced permease synthesis is inhibited in mutants which lack β-galactosidase. Therefore, it is clear that the inhibiting action of glucose is not at the level of the activity of either permease or galactosidase but at some other point in the series of reactions leading to the synthesis of either component.

The experiments described here are best understood in terms of the hypothesis that induced enzymes are those whose synthesis can be repressed (Neidhardt and Magasanik, 1956*a*, *b*; 1957; Vogel, 1957) by some derivative of the carbon source. The inducer relieves the repression. Certain very inhibitory carbohydrates, such as glucose or mannitol, are metabolized to yield high internal levels of repressor, whereas less inhibitory substances such as succinate and lactate give lower levels of repressor. The repressor-hypothesis with some qualifications will be used as the basis for the description of the data.

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However, in the specific case of the glucose-inhibition, this simple hypothesis must be qualified somewhat to analyze the data quantitatively. As a result this system appears to be quite complex. In an attempt at simplification, we have decided to divide this paper into two parts; in the first half we will present the general behavior of populations with respect to induction and inhibition under various conditions, and in the second half we will discuss the proposed theories of the mechanism.

MATERIALS AND METHODS

Most of the pertinent techniques have been discussed previously (Cohn and Horibata, 1959*a*, *b*; Rickenberg *et al.*, 1956). The details of the experiments are to be found with the figures.

Strains of bacteria. In addition to those previously described, three strains of *Escherichia coli* which produce β-galactosidase or permease constitutively were used.

Strain	*Y*	*Z*	*Growth on lactose*
ML308	+	+	+
ML3088	+	−	−
ML35	−	+	−

Y refers to the galactoside-permease and Z to the β-galactosidase itself.

Measurement of enzyme synthesis. The differential rate of synthesis, i. e., enzyme activity expressed as a function of bacterial growth, has been discussed in several papers (Cohn, 1957; Monod *et al.*, 1952).

EXPERIMENTAL RESULTS

Several parameters of the system. (1) Specificity of the inhibition:—In this section we will present the kinetics of formation of β-galactosidase in the presence of various carbon sources, metabolizable and nonmetabolizable. The kinetics of the response of an *E. coli* (Y+ Z+) to induction appears to depend upon (a) the rate of metabo-

lism of the carbon source to yield the actual inhibitor, (b) the state of adaptation of the cells to both the inhibitory carbon source and the inducer, and (c) the degree of inhibitory action of the carbohydrate without further metabolism (direct action).

Almost any sugar and several amino acids at a high enough concentration (10^{-1} to 10^{-2} M) inhibit the induced appearance of β-galactosidase in *E. coli* strain ML30 (Y+ Z+). However, at concentrations (10^{-4} M) where the inhibition by glucose and mannitol are still maximal, the other substances have no effect. Certain sugars (gluconate, ribose, xylose, mannose, and fructose) at low levels block β-galactosidase synthesis for short periods up to a maximum of one division, at which time the organisms escape the inhibition (figure 1). Since a concentration of 10^{-3} M sugar would support a growth of at least 40 μg bacterial N per ml, this escape is not due to the disappearance of the sugar by metabolism. *E. coli* strain ML30 will grow on all of the substances listed in figure 1 except methyl α-D-glucoside and sucrose.

Under the above conditions, the later decrease in the inhibition of the formation of enzyme in the presence of any one sugar may be interpreted in terms of the appearance of heterogeneity in the population (Cohn and Horibata, 1959*a*; Novick and Weiner, 1957). The heterogeneous response of a population to induction has been shown to be due to the permease (Cohn and Horibata, 1959*a*, *b*; Novick and Weiner, 1957). If the findings with glucose are extrapolated to other carbohydrate inhibitors, then the accelerating response of the culture to induction in the presence of inhibiting carbohydrates (figure 1) reflects this heterogeneity. In addition to the galactoside-permease, the heterogeneous response may be favored by a second factor. If some metabolic derivative of the carbon source is the principal inhibitory substance (repressor), then the chief variable affecting the kinetics of inhibition will be the level of repressor. This level will depend in part upon the rate of conversion of externally added carbohydrate to internal repressor. In the case of an inhibitory substance which is used adaptively, this rate goes from zero to maximum in the time it takes for the necessary enzymes to appear. Carbohydrates, e. g., glucose, which are metabolized by constitutive enzyme systems, are converted virtually

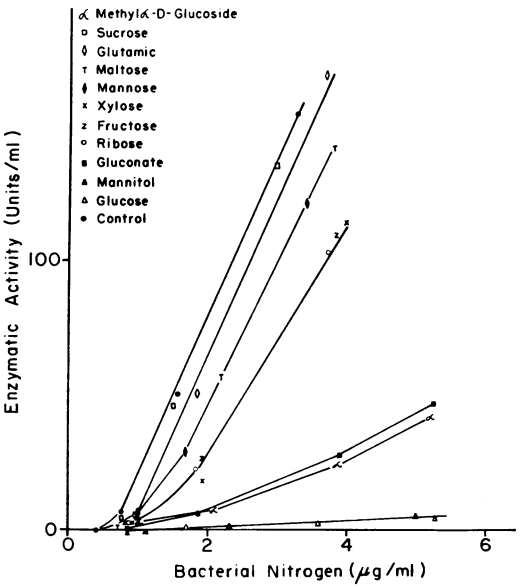


Figure 1. The effect of various substances at concentration of 10^{-3} M on the induction of β-galactosidase in *Escherichia coli* strain ML30 by 10^{-4} M methyl β-D-thiogalactoside (TMG). The inducer TMG and the various inhibitors were added simultaneously to the culture growing on succinate.

instantaneously to maximal levels of repressor. The consequences of these considerations can be illustrated with ribose which *E. coli* strain ML30 utilizes adaptively, but which *E. coli* strain B does not metabolize under any circumstances. Although the methyl β-D-thiogalactoside (TMG)-induced synthesis of β-galactosidase is blocked by glucose in noninduced *E. coli* strain B, ribose has no effect. On the other hand, the nature of the inhibition by ribose with *E. coli* strain ML30 depends upon whether or not the culture is preinduced with ribose or with TMG. If the culture is preinduced with ribose and then TMG is added, induced β-galactosidase synthesis is blocked much as in the case of an inhibitor such as glucose (figure 2). Conversely, if TMG is added first and the culture is preinduced for the galactoside-permease and the β-galactosidase, then ribose has no effect (figure 2). If both ribose and TMG are added simultaneously to a noninduced culture there is a race between two inducible systems, the one which handles the inhibitor and the one

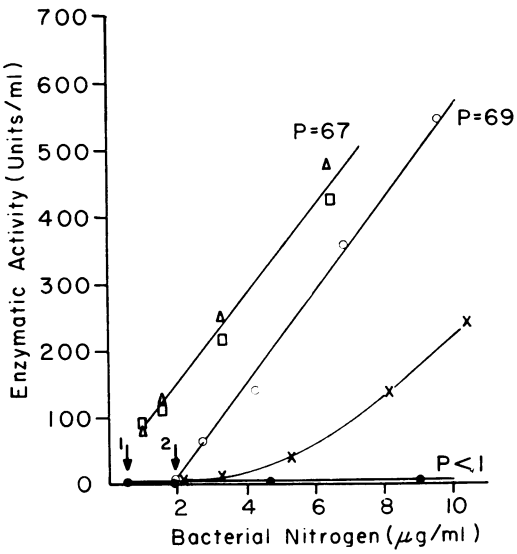


Figure 2. The ribose-methyl-β-D-thiogalactoside (TMG) interaction in strain ML30. To samples of a noninduced culture growing on succinate, (●——●) at *arrow 1*, ribose (2×10^{-3} M) was added, then at *arrow 2*, TMG (2×10^{-4} M) was added; (○——○) at *arrow 2*, TMG (2×10^{-4} M) alone was added; and (×——×) at *arrow 2*, TMG (2×10^{-4} M) and ribose (2×10^{-3} M) were added simultaneously.

To samples of a preinduced culture prepared by at least 3 divisions of growth in the presence of TMG, subsequently washed and put into exponential growth on succinate, (△——△) TMG (2×10^{-4} M) was added and (□——□) TMG (2×10^{-4} M) + ribose (2×10^{-3} M) were added.

which handles the inducer. Such a situation favors a heterogeneous response to induction since each cell might be expected to resolve this competition independently and differently. The resultant acceleration phase of the induction in the presence of ribose (as well as other carbohydrates, figures 1 and 2) can be simply interpreted in these terms.

The above discussion applies largely to metabolizable carbohydrates which are presumably converted as is glucose to a single inhibitor which interacts with the β-galactosidase system. Certain of these carbohydrates, either by virtue of their similarity in structure to galactosides or by any other means, might also have an inhibitory effect without being metabolized. This brings us to the third complication, that of direct action, which is illustrated by methyl α-D-glucoside (Wainwright, 1953) which enters the cell via a constitutive permease (Cohen and Monod, 1957) and is not metabolized as a carbon and energy source. This carbohydrate is not as good an inhibitor as glucose or mannitol since the culture relatively rapidly escapes inhibition at a concentration of 10^{-3} M, and at 3×10^{-4} M or below methyl α-D-glucoside is without effect. Preinduction with TMG (Cohn and Horibata, 1959*b*) not only renders the cells resistant to the inhibitory action of methyl α-D-glucoside (figure 3) but to all the substances listed in figure 1.

The action of methyl α-D-glucoside is not clear, nor is it certain that this derivative should be included in the description of diauxie-inhibitors. On the other hand, as we will discuss in detail later, the inhibition by glucose is only partially reversed by preinduction or by high inducer concentration. This leads to the qualification of the repressor hypothesis mentioned in the introduction, namely, that glucose is acting via two pathways: (a) indirectly as a metabolic inhibitory derivative (repressor), and (b) directly as unchanged glucose. Methyl α-D-glucoside

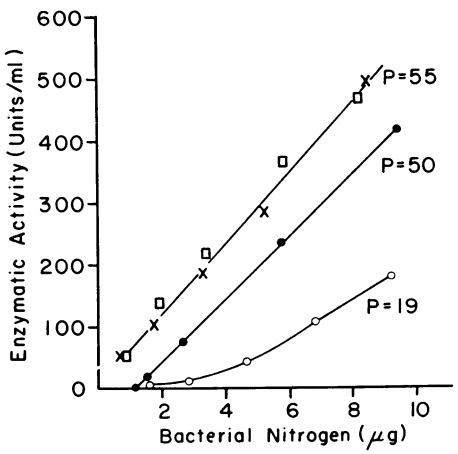


Figure 3. Methyl α-D-glucoside as an inhibitor of induction in strain ML30. To a noninduced culture growing on succinate was added, (●——●) methyl β-D-thiogalactoside (TMG) (10^{-4} M) and (○——○) methyl α-D-glucoside (10^{-3} M); after 2 hr of growth the culture was diluted into methyl α-D-glucoside (10^{-3} M) + TMG (10^{-4} M).

To a preinduced culture growing on succinate was added, (□——□) TMG (10^{-4} M) and (×——×) TMG (10^{-4} M) + methyl α-D-glucoside (10^{-3} M).

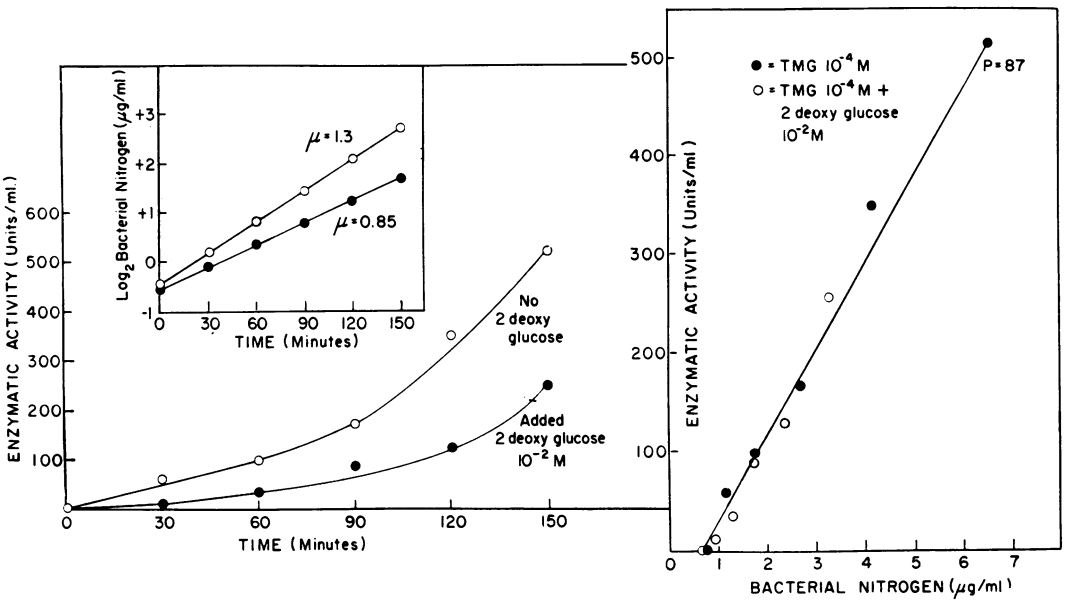


Figure 4. The effect of 2-deoxy glucose on induced β-galactosidase synthesis. To a portion of an *Escherichia coli* ML30 culture growing exponentially in succinate inorganic salts medium was added methyl β-D thiogalactoside (TMG) 10^{-4} M and to another portion was added 2-deoxy glucose, 10^{-2} M. (a) The time-activity curve with an insert of the growth curve. Growth rate = μ = divisions/hr. (b) The growth-activity curve (differential rate of synthesis).

looked at as a nonmetabolizable glucose analogue, might be reflecting the inhibitory action of glucose as such. We will return later to this question when the concentration relationships between inducer and inhibitor are considered.

In addition to methyl α-D-glucoside, there are sugar derivatives, such as 2-deoxyglucose, which markedly inhibit growth of *E. coli* on succinate but do not affect the differential rate of synthesis (figure 4).

(2) Anaerobic shock:—If one places an aerobically growing, glucose-inhibited culture (ML30) under anaerobic conditions, there is a lag in growth and at the resumption of growth, enzyme synthesis also begins despite the presence of glucose. This can be seen from the experiment described in figure 5. With succinate as sole carbon source, the switch to anaerobiosis causes a cessation of growth and of enzyme synthesis.

Anaerobic conditions alone are insufficient to establish enzyme formation in the presence of glucose. If a culture is first adapted to and growing exponentially on glucose under anaerobic conditions, the addition of inducer does not lead to enzyme formation (figure 6). It is the switch to anaerobic conditions which is effective. In terms of the preinduction effect described previously (Cohn and Horibata, 1959*b*; Monod, 1956; Novick and Weiner, 1957), one might hypothesize that during the conversion of metabolism from aerobiosis to anaerobiosis, there is a sufficient synthesis of Y so that the subsequent expected glucose-inhibition, either aerobic or anaerobic, is in large measure eliminated. This hypothesis is supported by the experiment (figure 7) showing that maintenance cannot be induced in ML3, the Y^- Z^+ cryptic strain. Upon making the cells anaerobic, there is a short burst of β-galactosidase synthesis which ceases as exponential anaerobic growth on glucose begins.

Since it is known (Cohn and Horibata, 1959*a*, *b*) that preinduction of the culture by aerobic growth on succinate in the presence of inducer leads to resistance to the inhibitory action of glucose and to a steady state of enzyme synthesis (maintenance), it might be asked whether preinduction under conditions of anaerobic shock leads to maintenance upon return to aerobiosis. Figure 8 illustrates the experiment showing that

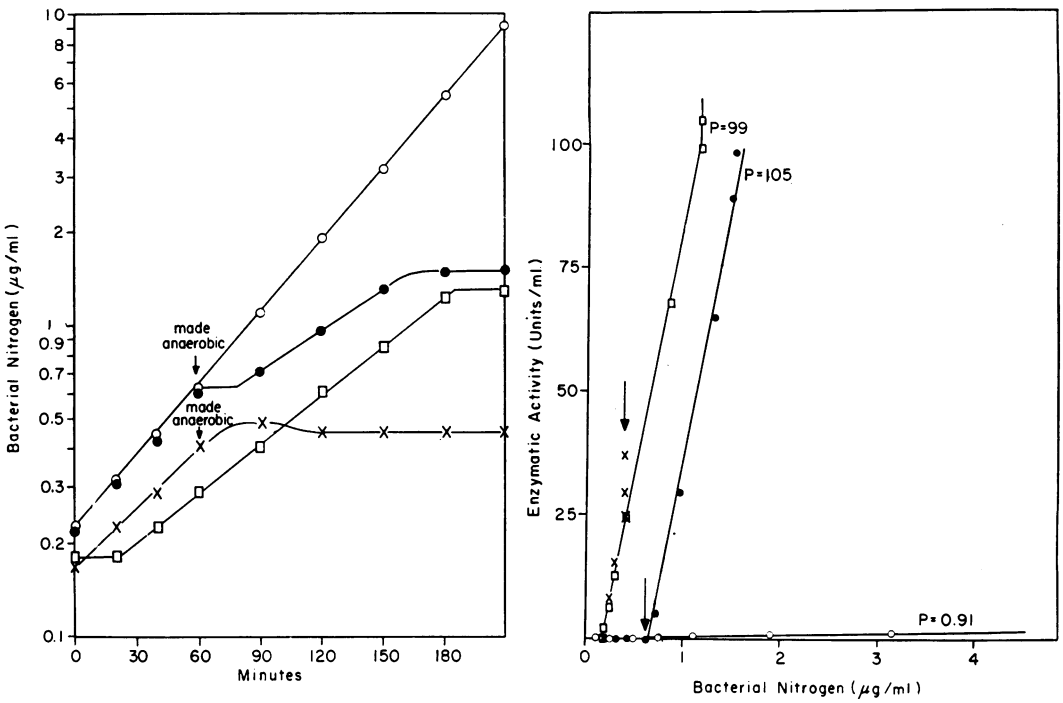
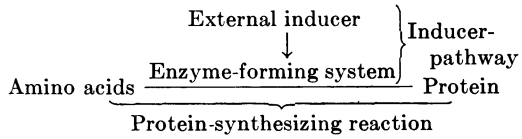


Figure 5. The effect of the change to anaerobic conditions upon the inhibition by glucose of β-galactosidase synthesis in *Escherichia coli* strain ML 30. *Left,* the growth curves for the cultures described below. *Right,* the differential rates of synthesis for above cultures. A noninduced aerobically growing culture is diluted into succinate medium 56 containing methyl β-D-thiogalactoside (TMG) (10^{-4} M) and, (X——X) no additions, aerobic, then anaerobic, curve 1; (O——O) glucose (10^{-3} M), aerobic, curve 2; (□——□) glucose (10^{-3} M), anaerobic, curve 3; and, (●——●) glucose (10^{-3} M), aerobic, then anaerobic, curve 4. At the times indicated by arrows the cultures represented by curves 1 and 4 were made anaerobic by bubbling through the culture 95 per cent N_2-5 per cent CO_2. These strains will not grow anaerobically on glucose unless CO_2 is added. Control experiments in which CO_2 was added to aerobically growing glucose inhibited cultures had no effect on the inhibition.

an anaerobic preinduction does lead to maintenance in aerobiosis and that this state is stable since it lasts through at least 27 generations (table 1).

Anaerobic shock permits enzyme induction not only in the presence of glucose, but also in the presence of other carbohydrate inhibitors such as gluconate and mannitol (figure 9). The anaerobic growth rate of the culture during this experiment is constantly increasing, yet the formation of enzyme as a function of bacterial mass becomes maximal and constant immediately.

Mechanism of glucose inhibition. We will be concerned here with the level at which the interaction of inducer and inhibitor takes place. The pathway of the induced synthesis of enzymes will be considered as consisting of two steps: (a) the inducer pathway whereby the externally added inducer reaches its site of action at the enzyme-forming system, and (b) the protein-synthesizing reaction involving the intermediates between amino acids and finished enzyme. In its simplest form (Cohn and Monod, 1953) one might represent the induction of a protein as shown in schema 1.



Schema 1

The first proposals, made some 10 years ago, as to the mechanism of the glucose effect, i. e., the interaction hypothesis (reviewed by Spiegel-

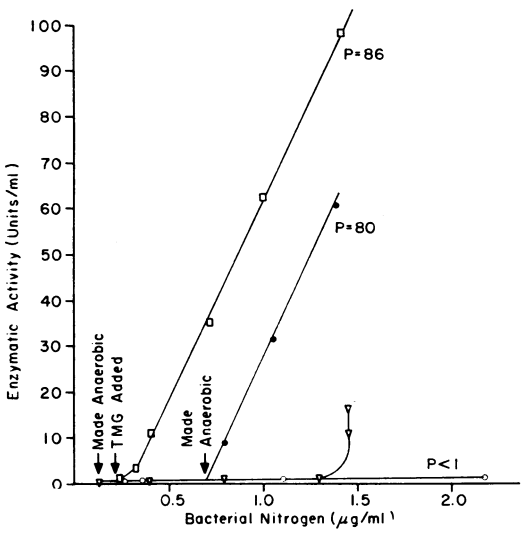


Figure 6. The effect of preadaptation to anaerobic conditions on the inhibition by glucose of methyl β-D-thiogalactoside (TMG) induction in *Escherichia coli* strain ML30 (Y^+Z^+). A sample of strain ML30 exponentially growing aerobically on succinate was placed (1) into glucose (10^{-3} M) and made anaerobic immediately by gassing with a 95 per cent N_2-5 per cent CO_2 mixture. When growth resumed, TMG (10^{-4} M) was added (▽——▽) (note that at the end of growth due to exhaustion of glucose some induction of Z occurs); (2) into TMG (10^{-4} M) and maintained aerobic (□——□); and (3) into glucose (10^{-3} M) and TMG (10^{-4} M) maintained aerobic (○——○). At the moment indicated, a sample was switched to anaerobiosis (●——●).

man, 1950), placed the action at the level of the protein-synthesizing reaction. More recently, the hypotheses (Cohn and Monod, 1953; Neidhardt and Magasanik, 1956*a*, *b*; 1957) have been that glucose acts at an early stage, i. e., on a step in the pathway between external inducer and its action in the enzyme-forming system.

(1) Interaction hypothesis:—This conception stemmed from an inquiry into the nature of the percursor of enzyme. It was proposed (Monod, 1941; Spiegelman, 1950) that different enzyme-forming systems competed for common building blocks. Under this interaction hypothesis, in the presence of glucose, the enzyme-forming systems which synthesized the glucose-induced enzymes used up the precursors which served also for other inducible systems. The result was an inhibition of synthesis of the latter enzymes.

There are several findings not satisfactorily explained by this hypothesis.

(a) Mutants which produce the β-galactoside-enzyme system constitutively are relatively resistant to the inhibitory action of glucose (figure 10). Even at concentrations as high as 10^{-1} M glucose, the constitutive synthesis of Y and Z is inhibited only 50 per cent, whereas the induced synthesis would be completely blocked at 10^{-4} M glucose. Formally speaking, the constitutive *E. coli* strains differ from the inducible strains in that constitutives in the absence of external inducer are not utilizing the inducer pathway. Since the inducible and constitutive enzymes are indistinguishable (Monod and Cohn, 1952), the above representation assumes that the protein-synthesizing pathway for the two strains are identical. If glucose acted at this latter level, constitutive synthesis should be blocked. This argument is applicable to each of the components of the β-galactoside system individually, for not only is constitutive Z synthesis by the Y^- mutant, ML35, resistant to the inhibition by glucose (figure 11*a*) but also constitutive Y synthesis by the Z^- mutant, ML3088 (figure 11*b*).

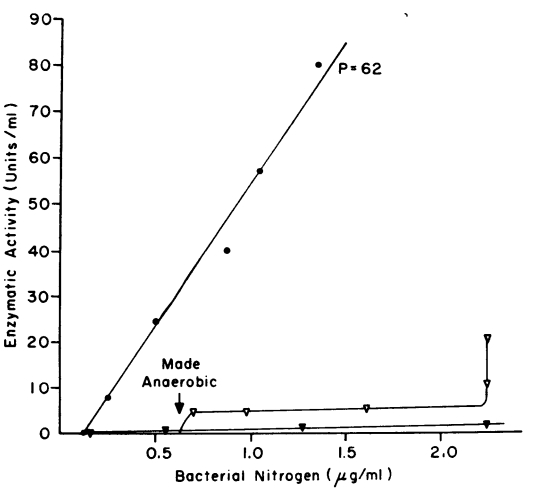


Figure 7. The effect of preadaptation to anaerobic conditions on the inhibition by glucose of methyl β-D-thiogalactoside (TMG) induction in *Escherichia coli* strain ML3 (Y^-Z^+). A sample of strain ML3 exponentially growing aerobically on succinate was placed (1) into TMG (8×10^{-4} M), maintained aerobic (●——●) and (2) into TMG (8×10^{-4} M) + glucose (2×10^{-3} M), maintained aerobic (▼——▼). At the moment indicated, a sample was switched to anaerobiosis (▽——▽).

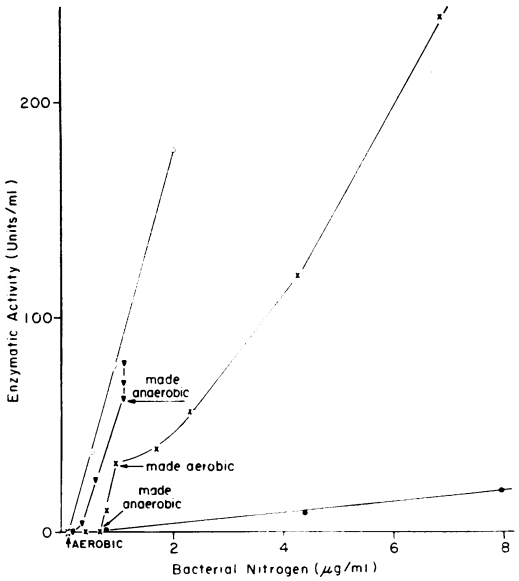


Figure 8. The establishment of maintenance in *Escherichia coli* strain ML30 as a result of anaerobic treatment. An aerobically growing culture is diluted into succinate medium 56 containing methyl β-D-thiogalactoside (TMG) (10^{-4} M) and, (▼——▼) no additions, aerobic, culture 1; (○——○) glucose (10^{-3} M), anaerobic, culture 2; (×——×) glucose (10^{-3} M), aerobic, culture 3; and (●——●) glucose (10^{-3} M), aerobic, culture 4.

At times indicated by arrows, the cultures 1 and 3 were made anaerobic as described in figure 5. After 30 min anaerobic induction, the culture described by curve 3 was made aerobic.

(b) The addition to the medium of amino acids, peptones, yeast extract, nucleosides, purine and pyrimidine bases, vitamins, or CO_2 did not reverse the glucose inhibition of β-galactosidase synthesis. If anything, these additions increase the inhibitory power of glucose. This finding confirms that of Neidhardt and Magasanik (1957) with the histidinase system of *Aerobacter aerogenes*. If there were a sudden depletion by glucose of common intermediates in protein synthesis, the above additions might be expected to relieve the shortage and allow β-galactosidase formation, particularly since they increase the growth rate significantly.

(c) The interaction hypothesis does not predict maintenance which would be specifically dependent on the presence of the Y system (Cohn and Horibata, 1959*b*; Novick and Weiner, 1957), unless it be assumed that the permease itself is the intermediate which is common to the synthesis both of the β-galactosidase and of the glucose-induced enzymes, and which is competed for by the two systems. This is known to be incorrect since there are mutants (ML3) which grow on glucose as sole carbon source and which, lacking galactoside-permease, make β-galactosidase. The induced synthesis of β-galactosidase is inhibited by glucose in these cryptic strains (Herzenberg, 1958).

(2) Interference by glucose with inducer action:—As the interaction hypothesis became less tenable, the second class of hypotheses suggested (Cohn and Monod, 1953; Neidhardt and Magasanik, 1956*a*, *b*; 1957) was that glucose acted at the level of inducer action, that is on the pathway between external inducer and the internal complex between the inducer or its derivative and the enzyme-forming system. The first of the hypotheses in this category was that glucose or a metabolic derivative of it blocked the penetration of the inducer into the cell. This hypothesis was proved wrong by Neidhardt and Magasanik

TABLE 1

Maintenance induced by anaerobic shock

Conditions	No. of Divisions	Differential Rate of Synthesis	
		Y	Z
Noninduced			
TMG (methyl-β-D-thiogalactoside)	27	0.61	82
TMG + glucose, aerobic	27	<0.02	0.07
TMG + glucose, anaerobic	27	0.28	51
TMG + glucose, anaerobic, 2 hr, then aerobic	27	0.33	43
Preinduced			
TMG + glucose, aerobic	27	0.39	52

A noninduced and preinduced culture of ML30 were diluted to the level of 1 organism per ml into succinate medium at 37C and treated as indicated above. 24 to 29 hr later, when the cultures had reached a density between 1×10^8 and 3×10^8 bacteria per ml, they were analyzed. The concentration of TMG was 10^{-4} M and of glucose was 2×10^{-3} M. See figure 2 for technique of aerobic preinduction.

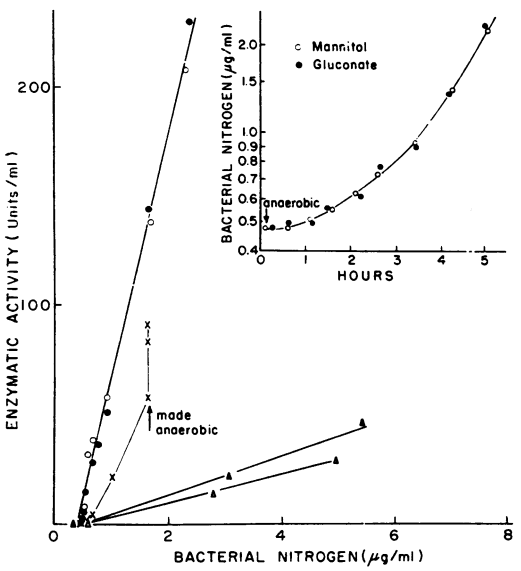


Figure 9. The effect of anaerobic shock on the inhibition by mannitol and gluconate. *Escherichia coli* strain ML30 growing aerobically in succinate-salt medium was placed into (×——×) methyl β-D-thiogalactoside (TMG) (10^{-4} M), culture 1. (○——○) TMG (10^{-4} M) + mannitol (2×10^{-3} M) anaerobic, culture 2; (△——△) TMG (10^{-4} M) + mannitol (2×10^{-3} M) aerobic, culture 3; (●——●) TMG (10^{-4} M) + gluconate (2×10^{-3} M) anaerobic, culture 4; and (▲——▲) TMG (10^{-4} M) + gluconate (2×10^{-3} M) aerobic, culture 5. Culture 1 after induction had progressed was made anaerobic. The insert illustrates the anaerobic growth of the cultures on mannitol and gluconate.

(1957) for the histidinase system, and for the β-galactoside system by showing that lactose is metabolized by induced cells in the presence of glucose, and by direct measurements of inducer penetration (Cohn and Horibata, 1959*b*). Therefore, if glucose interferes with the metabolism of the inducer, it must be at a stage after the action of the Y system. This point is again supported by the observation that cryptic strains, in which the inducer metabolism can be made to by-pass the Y system, are glucose-inhibited (Herzenberg, 1958).

These considerations lead to the repressor hypothesis, namely, that glucose is converted by inducible cells to a specific repressor which interacts with the enzyme-forming system of the galactosidase and the permease. The most obvious kind of repressor which can be postulated is a galactoside which would be an analogue of the inducer, TMG. One model for the behavior of such a substance would be phenyl β-D-thiogalactoside (TPG) which has been shown (Cohn and Monod, 1953) to be a competitive inhibitor of the induction by TMG, the interaction presumably taking place at the level of the enzyme-forming system (Herzenberg, 1958). This simple inducer-analogue hypothesis predicts that any procedure which increases the internal inducer concentration should diminish the extent of the glucose inhibition. One such way is preinduction and as has been shown (Cohn and Horibata, 1959*b*), concomitant with the appearance of the permease, the induced synthesis of Y and Z becomes resistant to glucose. However, a more critical test would be the demonstration of a competitive relationship between inducer and glucose. The effect of concentration of TMG and glucose on

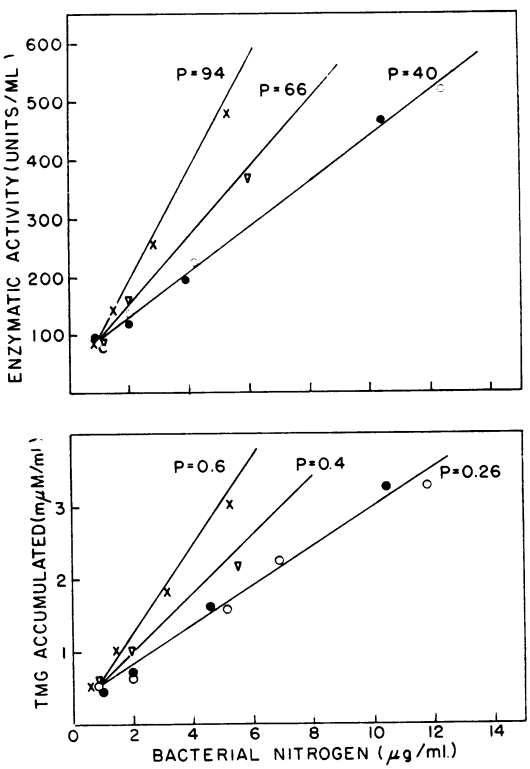


Figure 10. The effect of glucose on the constitutive formation of Y and Z by the mutant ML 308 (Y^+ Z^+) growing on succinate. (×——×) = no additions; (▽——▽) = glucose (10^{-3} M); (●——●) = glucose (3×10^{-3} M); and (○——○) = glucose (10^{-2} M).

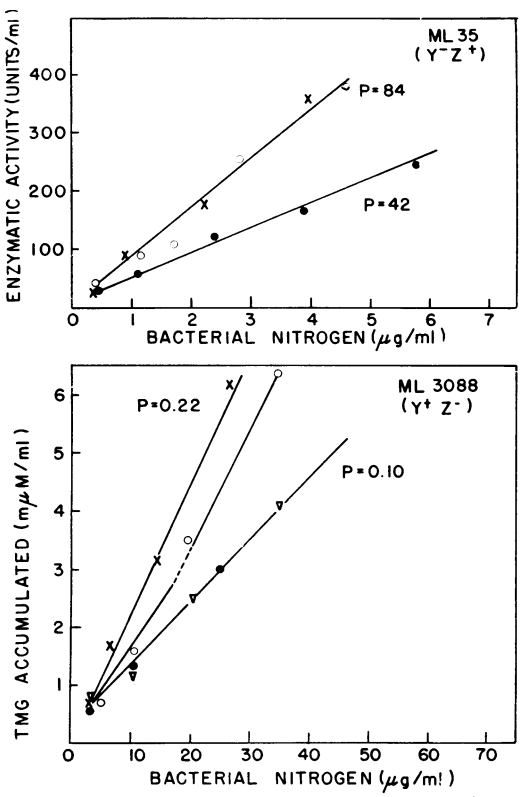


Figure 11. Inhibition by glucose of the constitutive synthesis of Z in the absence of Y (*upper graph*) and Y in the absence of Z (*lower graph*). Strain ML35 (Y^-Z^+): (×——×) = no additions; (○——○) = glucose (10^{-3} M); and (●——●) = glucose (10^{-2} M). Strain ML3088 (Y^+Z^-): (×——×) = no additions; (○——○) = glucose (10^{-3} M); (●——●) = glucose (3×10^{-3} M); and (▽——▽) = glucose (10^{-2} M).

the differential rate of synthesis by noninduced *E. coli* strain ML30 has been studied previously (Cohn and Horibata, 1959*b*) with the finding that at a given glucose concentration (10^{-3} M) increasing the TMG concentration led to a more rapid induction, but that the response of the population was heterogeneous. The previous paper (Cohn and Horibata, 1959*b*) also pointed out that with the preinduced cells there was a residual inhibition by glucose at 10^{-3} M which was not reversed by preinduction and continued growth in the presence of TMG at 10^{-4} M. No effect on the residual inhibition is observed by increasing the concentration of TMG even at low glucose concentration (10^{-4} M) (table 2).

The interaction of TMG and glucose is essentially noncompetitive in the preinduced culture. Since the glucose could have two actions (via a derivative and directly), and since the concentration of inducer in the cell (approximately 10^{-1} M) depends primarily on the level of permease and not on external inducer concentration above the saturating concentration of 3×10^{-4} M, these results are not a contradiction of the analogue hypotheses.

Herzenberg (1958) has carried out a careful analysis of the kinetics of the glucose-inducer interaction using cryptic *E. coli* strains and has found a distinctly noncompetitive relationship between inducer and glucose. Since his work was carried out with strains of *E. coli* which had been selected on maltose through many generations, and since the carbon source during the experiments was maltose (which is metabolized to glucose), it was decided to repeat this work under the present conditions.

For the reanalysis of this question (table 3) the more efficient inducer isopropyl β-D-thiogalactoside (IPTG), studied by Herzenberg, was employed instead of TMG. Furthermore, this experiment (performed some 2 years after all the others described here) was carried out using strains which had undergone such a prolonged selection on succinate that the induction of β-galactosidase synthesis was only weakly inhibited by glucose, unless a passage through glucose was carried out. Even then these strains were more resistant than the previously described organisms.

TABLE 2

Effect of methyl β-D-thiogalactoside (TMG)/glucose ratios on preinduced Escherichia coli strain ML30

TMG Conc	Glucose Conc (M):			
	10^{-3}	5×10^{-4}	10^{-4}	0
M				
10^{-2}	27	29	38	60
10^{-3}	27		42	64
10^{-4}	27	24	38	60
3×10^{-5}		24	33	55

A maximally induced culture was diluted into succinate medium 56 containing the above mixtures of TMG + glucose. The figures are the differential rate of synthesis, expressed as units of enzyme per μg bacterial nitrogen.

TABLE 3

Effect of inducer concentration on the inhibition by glucose by noninduced Escherichia coli

IPTG*	Glucose (M)							
	0		10^{-4}		10^{-3}		10^{-2}	
	ML30	ML3	ML30	ML3	ML30	ML3	ML30	ML3
M								
10^{-2}	70	58	76 (<1%)	52 (11%)	14 (80%)	19 (67%)	<0.1 (>99%)	2.9 (95%)
10^{-3}	73	47	62 (15%)	36 (24%)	3.6 (94%)	8.9 (81%)	<0.1 (>99%)	<0.1 (>99%)
10^{-4}	68	9.3	15 (78%)	2.9 (69%)	<0.1 (>99%)	<0.1 (>99%)	<0.1 (>99%)	<0.1 (>99%)

E. coli strains ML30 (Y^+ Z^+) and ML3 (Y^- Z^+) growing in succinate were placed into the above indicated concentrations of glucose and IPTG. After 15 min the cultures were analyzed and the differential rate of synthesis calculated. The figures in parentheses are the percentage inhibition calculated with respect to the corresponding control with no glucose added.

* Isopropyl β-D-thiogalactoside.

At a low glucose concentration (10^{-4} M) the increasing inducer level leads to restoration of maximum activity. However, at 10^{-3} M glucose and above, there appears to be a residual inhibition quite resistant to increased inducer concentration. This apparent noncompetitive aspect of glucose action is not a formal argument contradicting the analogue hypothesis, since it can be accounted for by any one or combination of the following assumptions: (a) that the concentration of the inhibitor derived from the glucose has a very high affinity for the enzyme-forming system, (b) that glucose has two distinct inhibitory effects on this system, one competitive via a derived inhibition metabolite, and the other noncompetitive and direct (see discussion of figure 3), or (c) that the glucose-derived inhibitor has a specific but in part noncompetitive effect on induction.

DISCUSSION

Ever since Monod's (1941) study of diauxie, it has been clear that a large number of structurally unrelated substances could each inhibit the induced synthesis of an equally large number of apparently unrelated enzymes (for bibliography see Cohn and Horibata, 1959*b*). These findings are conveniently interpreted as meaning that (a) for a given induced enzyme the various diauxic inhibitors are metabolized to a common identical repressor which has direct action, and (b) for a given diauxic inhibitor the various induced enzymes are each repressed by a different specific repressor derived via the intermediate metabolism of the diauxic inhibitor. This "feed-back" model of glucose action was proposed by Magasanik (1957) and Neidhardt and Magasanik (1956*a*, *b*; 1957) to account for their interesting findings with the histidinase system. Were it not for the complication due to the noncompetitive aspect of the glucose inhibition, all of the findings could be understood in terms of the repressor hypothesis with the further specification that the repressor is a galactose analogue (of high or low molecular weight). To account for the noncompetitive aspect, the inhibitory behavior of glucose in the β-galactoside system is assumed to be complex, comprising two actions: a weak direct one, shown at high concentrations and noncompetitive in nature; and a strong indirect one, caused by a metabolic derivative, apparently competitive in nature and shown at low glucose concentrations. The argument that glucose itself is metabolized to a repressor is supported by the observation that nonmetabolizable derivatives of glucose, e.g., 2-deoxy glucose, 3-methyl glucose, methyl α- or β-D-glucoside, sucrose, cellobiose, phenyl β-D-thioglucoside, have little effect compared to glucose on induced β-galactosidase synthesis. Whatever action these analogues do have is attributable to an inhibition by the unchanged substance.

The indirect action implies that the difference between glucose or mannitol, which are excellent inhibitors, and ribose or fructose, which are not as effective, is that for the strains of *E. coli* used here the steady state concentration of the metabolically derived repressor in the cell is

higher for the former than for the latter substances. The rate of metabolism of a diauxic inhibitor to a repressor is also clearly dependent on the strain, for by repeated selection on succinate one can isolate strains which are increasingly resistant to the inhibition by glucose. These organisms grow well on glucose although they metabolize it aerobically at one half the rate of glucose-maintained strains, the implication being that resistance to inhibition and reduced metabolism of glucose are related. Thus, on the one hand, carbon sources differ quantitatively as inhibitors of induction for a given organism and, on the other hand, various organisms respond differently to given inhibitors of induction depending upon their rates of metabolism.

At the extreme end of this spectrum are succinate, lactate, glycerol, malate, acetate, which for *E. coli* strains ML, B, and K12 are apparently noninhibitory in our experiments. However, under other conditions (Mandelstam, 1957) an apparently noninhibitory substance such as succinate becomes as inhibitory as glucose to induction. The suggestion derived from this picture is that all the carbon sources are metabolized to repressors and that the induced enzyme is simply in a repressed state. Inhibitory carbon sources are those which are converted to such a surplus of repressor that induction by substances added to the medium is decreased.

The above speculation raises the question as to why glucose does not have an inhibitory effect on constitutive β-galactosidase synthesis comparable to its effect on induced synthesis? The answer to this depends upon which hypothesis concerning constitutivity is accepted, "the generalized induction hypothesis" (Cohn and Monod, 1953) or the "generalized repression hypothesis" (Hogness, 1959; Pardee *et al.*, 1958; Vogel, 1957).

Since present evidence favors the interpretation that inducible enzymes are those which are repressed in synthesis (Pardee *et al.*, 1958) we will discuss this point of view further. The resistance of constitutive synthesis to the inhibition by glucose implies under the repression hypothesis that there is a block in the specific pathway of metabolism from glucose to the repressor or of the synthesis of the β-galactoside-enzyme system.

In general our findings are more simply interpreted in terms of a repression hypothesis because no special assumptions as to the pathways of metabolism have to be made to account for the difference between apparently noninhibitory and inhibitory carbon sources. The difference is purely quantitative with noninhibition being the basal inhibitory level.

It is interesting that according to the repression hypothesis the resistance of a preinduced cell and of a constitutive cell to the glucose inhibition are caused by two distinct mechanisms, high internal inducer concentration in the preinduced cell and lack of repression in the constitutive cell.

Like glucose, TPG (Cohn and Monod, 1953) is a very poor inhibitor of constitutive synthesis. These two substances have a marked inhibitory effect on induced synthesis even in cryptic organisms lacking the galactoside permease (Herzenberg, 1958). Under the repression hypothesis, if it is the metabolism of glucose to a galactoside-analogue repressor which is blocked, then TPG, which under the simplest hypothesis is like the repressor end product of glucose metabolism, should inhibit. Since it does not, the attractive hypothesis that the repressor actually is a galactoside analogue has little experimental support.

One point deserves comment; there are conditions under which the differential rate of synthesis increases sharply (figures 5 to 9). This curious observation, which was first made by Rickenberg and Lester (1955), and termed by them preferential synthesis, crops up repeatedly whenever a culture, in the presence of inducer, begins to starve on a carbon source either because it is limiting or because it becomes unavailable metabolically, e. g., succinate during conversion to anaerobiosis.

In terms of the repression hypothesis, the concentration of inhibitor derived from succinate or glucose would become so low that the TMG-inducer would exert an explosive inductive effect.

This interesting phenomenon of preferential synthesis deserves a detailed experimental analysis.

SUMMARY

The complex kinetics of β-galactosidase synthesis resulting from the interaction of carbohydrate inhibitors and inducers has been analyzed in terms of the hypothesis that the different carbon sources are all metabolized to one given inhibitor which competes with inducer at the level of the enzyme forming system.

The data discussed include: (a) the effect of

metabolizable and nonmetabolizable sugars on both induced and constitutive synthesis; (b) the concentration relationships between glucose and inducer, and (c) the role of aerobiosis.

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