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Phenomenon of Transient Repression in *Escherichia coli*

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ABSTRACT

PAIGEN, KENNETH (Roswell Park Memorial Institute, Buffalo, N.Y.). Phenomenon of transient repression in *Escherichia coli*. J. Bacteriol. **91**:1201–1209. 1966.—A family of mutants has been obtained in *Escherichia coli* K-12 in which β-galactosidase is not inducible for approximately one cell generation after the cells are transferred to glucose from other carbon sources. After that period, the enzyme can be induced at the level appropriate to glucose-grown cultures of the parent cells. Among a wide variety of carbon sources, the only one capable of eliciting a state of transient repression is glucose. Conversely, transient repression occurs when cells are transferred to glucose from any of a variety of other carbon sources. The only exceptions to this so far discovered are lactose, gluconate, and xylose. Susceptibility to transient repression in mutants can also be induced in glucose-grown cells by a period of starvation. Mutant cells which have become susceptible to transient repression lose susceptibility in the presence of glucose only when they are under conditions which permit active protein synthesis. The presence of an inducer of β-galactosidase is not required during this time, nor does pre-induction for β-galactosidase diminish the susceptibility of mutants. At least two other catabolite repression-sensitive enzymes (galactokinase and tryptophanase) are also sensitive to transient repression, and the two phenomena are probably related. The absolute specificity of glucose and the pattern of response seen after growth in different carbon sources suggest that the endogenous metabolite which produces these repressions is far more readily derived from glucose in metabolism than it is from any other exogenous carbon source.

When cultures of certain strains of *Escherichia coli* are transferred from a medium containing glycerol to one containing glucose and an inducer of β-galactosidase, there is no delay in the resumption of growth, but the cells do not make detectable amounts of β-galactosidase for a period of approximately one generation. Such cultures then recover the ability to form enzyme. This phenomenon, termed transient repression, was first detected in a galactose-negative mutant of *E. coli* K-12. It was subsequently found to be characteristic of a group of strains descended from a common ancestor, and to be absent from other strains. Among the strains exhibiting transient repression, the expression of this character is independent of galactose fermenting ability. In addition to β-galactosidase, at least two other enzymes, galactokinase and tryptophanase, are subject to transient repression, implying that a factor common to the regulation of all three has been altered in sensitive cells.

In bacteria, the ability of one carbon source to inhibit the formation of an enzyme system required in the metabolism of another has been known for some time. First described by Epps and Gale (2) and Gale (3) as the "glucose effect," it has been extended by Neidhardt (7) and Magasanik (5) under the term "catabolite repression" to include effects of all carbon sources. They suggested that the relative ability of different nutrients to exert repression is a function of the rate at which they contribute to the common metabolic pool. The behavior of transient repression mutants suggests that this phenomenon is a special case of catabolite repression, and that any enzyme sensitive to catabolite repression should also be sensitive to transient repression.

Transient repression mutants may therefore be useful in the study of the mechanisms of the glucose effect, in particular, in the identification of the endogenous or natural repressor common to these various systems. The term repressor is

here used in its original sense (12) and refers to those small molecules whose internal concentrations serve to regulate the levels of repressible enzymes. A comparison of the ability of various carbon sources to participate in transient repression indicates that the internal metabolite effective in catabolite repression is either glucose itself or a close metabolic product.

MATERIALS AND METHODS

Culture conditions. The synthetic medium used (M95) was identical with the M9 medium of Anderson (1), except that the phosphate and nitrogen concentrations were one-fifth of their original values. Carbon sources were present at 0.1% concentration, unless noted otherwise. Cultures were grown at 37 C under active aeration. Cell growth is expressed as the relative increment A_t/A_o where A_t and A_o are the absorbancies at 550 mμ in a Zeiss spectrophotometer at times t and o. Isopropylthiogalactoside (IPTG) at 5×10^{-4} M, D(+) fucose at 0.1 M, and tryptophan at 2.1×10^{-3} M were used as inducers for β-galactosidase, galactokinase, and tryptophanase, respectively.

Enzyme measurements. Galactokinase and β-galactosidase were assayed as described previously (10). Tryptophanase was assayed by a modification of the method of Pardee and Prestidge (11). Sample cultures were centrifuged, and the cells were resuspended in water. Samples of the resuspended cells (0.1 ml) were then lysed by the addition of 0.10 ml of a two-thirds dilution of the lysis mixture [lysozyme-tris(hydroxymethyl)aminomethane(Tris) - ethylenediaminetetraacetic acid (EDTA)] used for the other two assays, and 5 min later 0.10 ml of substrate (containing 2.5 mg/ml of L-tryptophan plus 75 μg/ml of pyridoxal phosphate in 0.054 M KCl) was added. After 60 min of incubation at 37 C, the reaction was stopped by heating the tubes at 100 C for 2 min. The amount of indole formed in the reaction was determined by adding 1.0 ml of Ehrlich's reagent, allowing 10 min for color development, and measuring the absorbance at 570 mμ. A unit of enzyme is defined as that amount forming 1 μmole of indole per min. Enzyme activity was not strictly proportional to cell concentration during assay. Doubling the number of cells of a given sample that were taken for assay reduced the specific activity by about 20%. The addition of uninduced cells had no effect. For the induction curves reported, cultures were started at similar cell densities and were resuspended after centrifugation to constant cell density.

To facilitate comparison of induction curves, enzyme activities are expressed as $(E_t - E_o)/A_o$ where E_t and E_o are the amounts of enzyme per milliliter of culture present at times t and o, respectively.

Phenotypic testing. An abbreviated test for the determination of the transient repression phenotype was based upon the use of overnight cultures. Strains were grown to saturation for 18 hr in M95 with 0.2% glycerol as carbon source. The cells were harvested and resuspended to a cell density of 2×10^8 to 4×10^8 per milliliter in fresh medium containing IPTG and either 0.2% glycerol or glucose as carbon source. Growth and enzyme induction were determined after 2 hr of aeration. When treated in this manner, wild-type cells growing in glucose form 30 to 50% as much enzyme per unit growth in glucose as they do in glycerol; strains sensitive to transient repression form only 5 to 10% as much.

RESULTS

Transient repression phenomenon. The phenomenon of transient repression is defined by the experiment described in Fig. 1. A culture of a mutant strain sensitive to transient repression (TR^s) was grown in glycerol, and the cells were harvested and transferred to a fresh medium containing an inducer of β-galactosidase, but with glucose rather than glycerol as the carbon source. Growth began immediately; however, the cells did not make β-galactosidase for a period of approximately one generation. It is this lag in the initiation of enzyme synthesis which is termed transient repression. As the control experiments of Fig. 1 show, transient repression does not occur when TR^s mutants are placed in a fresh medium with glycerol as the carbon source, or when a wild-type culture of *E. coli* K-12 (TR^+) is transferred from glycerol to glucose.

When TR^s cultures are grown in glycerol and

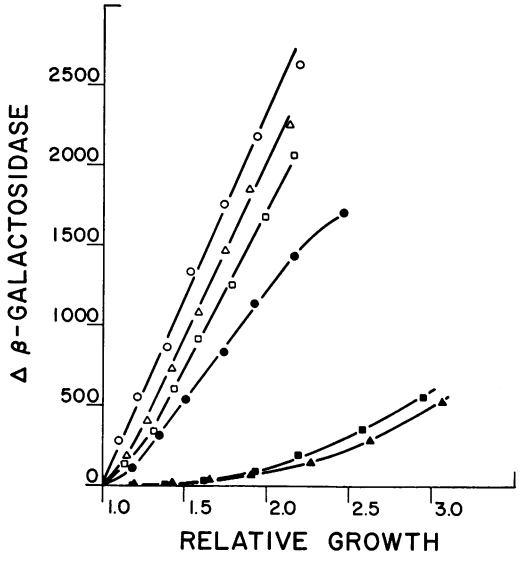

FIG. 1. *Transient repression character. Cultures grown to 2 × 10⁸ per milliliter with glycerol as carbon source were harvested and resuspended at the same density in fresh medium containing IPTG and either glycerol (open symbols) or glucose (closed symbols) as carbon sources. Growth and β-galactosidase activity were then followed. The cell strains were W-3110 TR^+ gal^+ (○, ●), W-3350 TR^s gal K^-T^- (□, ■), and Q22 TR^s gal^+ (△, ▲).*

transferred to glucose, they eventually recover the capacity to form β-galactosidase, and then do so at a normal rate. This is illustrated in Fig. 2, which examines a much longer growth period. In this case, TR^s cells were unable to make enzyme for one generation after transfer from glycerol to glucose. They then recovered and began making enzyme at the same rate as cells which had been grown in glucose throughout the experiment.

Control experiments indicate that transient repression represents an inhibition of enzyme formation rather than any interference with enzyme assay. Enzyme activity remained proportional to the number of cells assayed at all stages of induction, and TR^s lysates prepared from cells at all stages of growth had no effect on the enzyme assay of TR^+ cells.

Pre-induction of TR^s mutants. The results described in Fig. 2 also demonstrate that transient repression affects the maintenance as well as the initiation of the induced state. TR^s cells which had already been induced to a high level of β-galactosidase activity at the start of the experiment by prior growth in glycerol plus inducer for several generations were as sensitive to transient repression as cells which had not been previously exposed to inducer.

Susceptibility of tryptophanase and galactokinase to transient repression. The sensitivity to transient repression of enzymes other than β-galactosidase has been examined. In particular, the inductions of tryptophanase by tryptophan and of galactokinase by fucose, were studied. [Galactokinase is not formed in glucose cultures at the concentrations of fucose that are customarily used for induction in glycerol culture. The enzyme can be induced in the presence of glucose, however, at much higher concentrations of fucose (Williams and Paigen, *unpublished data.*] Figures 3 and 4 show the induction curves obtained when TR^+ and TR^s cultures were pregrown in either glucose or glycerol and then transferred to media containing glucose and the appropriate inducer.

In the case of tryptophanase, there was a relatively slow but steady formation of the enzyme when either TR^+ or TR^s cells were induced in glucose after pregrowth in glucose. However, after pregrowth in glycerol, TR^+ cells showed an initial very rapid burst of enzyme formation which eventually subsided to the rate of induction seen in glucose-adapted cells. In contrast, TR^s cells formed very little tryptophanase after transfer from glycerol to glucose culture for at least one generation, and often longer.

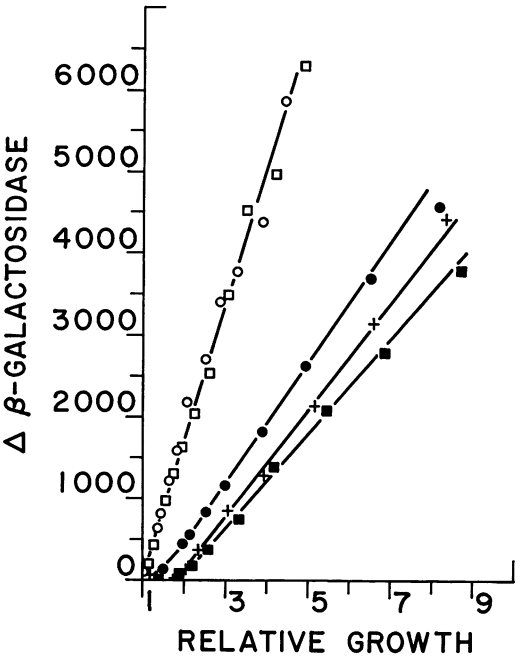

FIG. 2. *Recovery after transient repression. Cultures of TR^s cells (Q22) were grown to 2 × 10^8 per milliliter, harvested, and resuspended at 0.7 × 10^8 per milliliter in fresh medium containing IPTG. Conditions were pregrowth in glucose and transfer to glycerol (○), pregrowth in glycerol and transfer to glycerol (□), pregrowth in glucose and transfer to glucose (●), pregrowth in glycerol and transfer to glucose (■), pregrowth in glycerol + IPTG and transfer to glucose (×). Subsequent growth and β-galactosidase formation were followed.*

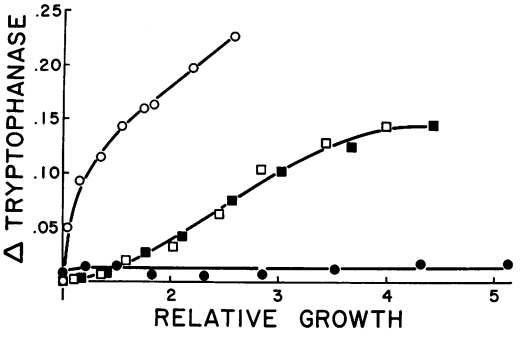

FIG. 3. *Sensitivity of tryptophanase to transient repression. Cultures of TR^+ (W-3110) and TR^s (Q22) cells were grown to 2 × 10^8 per milliliter, harvested, and resuspended in fresh medium containing 2.1 × 10^{-3} M tryptophan and 0.1% glucose. Conditions were pregrowth in glycerol (○, ●) or pregrowth in glucose (□, ■). Subsequent growth and tryptophanase formation were followed. Open symbols refer to TR^+, closed symbols to TR^s.*

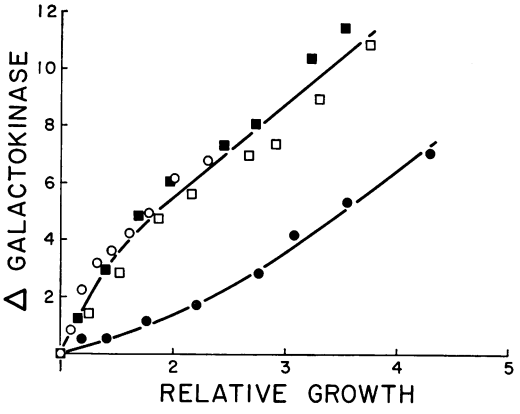

FIG. 4. *Sensitivity of galactokinase to transient repression. The experimental protocol and symbols are the same as for Fig. 3 except for the replacement of tryptophan by 0.1 M fucose. The ordinate is expressed in units of 10^3.*

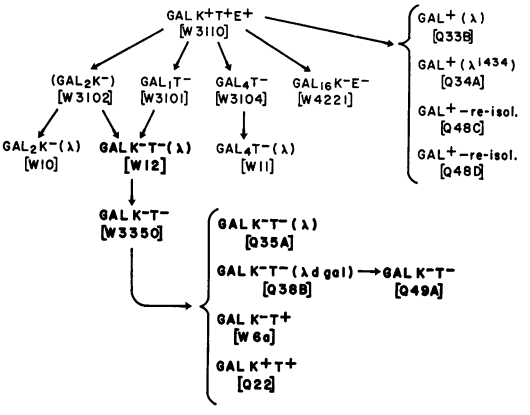

FIG. 5. *Family tree of TR^s mutants. TR^s strains are indicated in bold type, TR$^+$ in light face. The detailed history of the strains and isolation procedures are given in Table 1.*

When the induction of galactokinase was examined, a similar result was obtained. Either TR$^+$ or TR^s cultures formed enzyme without an appreciable lag after pregrowth in glucose when exposed to the inducer in glucose. TR$^+$ cells, after pregrowth in glycerol and exposure to inducer in glucose, were fully inducible. They occasionally showed an additional small burst of enzyme formation, analogous to that seen with tryptophanase. The magnitude of this additional burst was quite variable and is not present in the data of Fig. 4. TR^s cells showed a phase of transient repression similar to that seen with β-galactosidase and tryptophanase.

It thus appears that for both of the other enzymes studied, tryptophanase and galactokinase, there is an initial period of repression, analogous to that seen with β-galactosidase, which is found with TR^s but not TR$^+$ cells after transfer from a glycerol to a glucose medium.

Familial relationship of TR^s mutants. Transient repression was originally detected in a galactose-negative mutant (W-3350) carrying defects in both the galactokinase and galactose-1-phosphate-uridyl transferase genes. The familial relationships of this strain are indicated in Fig. 5, and the properties of its various relatives are listed in Table 1. Each of these strains has been diagnosed as TR^s or TR$^+$ by the abbreviated phenotypic testing procedure described under Materials and Methods. TR^s strains are indicated in bold type. The expression of the transient repression character was independent of the galactose-fermenting ability of the cultures, and its original association with the *gal*$^-$ character is considered to be fortuitous.

From the family tree, it would appear that the original mutation from TR$^+$ to TR^s occurred during the isolation of the strain *gal* K$^-$T$^-$(λ) (W-12). All descendants of this strain are TR^s. This double mutant was constructed by transduction via λ dg of the T$^-$ gene into the K$^-$ strain by J. J. Weigle. The first isolate was lysogenic for λ (W-12). The nonlysogenic strain K$^-$T$^-$ (W-3350) was subsequently obtained by him after exposure of the lysogenic strain to high ultraviolet dosage.

Among the strains tested, the TR^s phenotype remained stable during lysogenization and spontaneous curing for phages carrying λ immunity, and after both lysogenic and stable transduction from the *gal*$^-$ to the *gal*$^+$ condition. Other *gal*$^-$ mutants derived from the wild-type parent (W-3110) remained TR$^+$. It thus appears that the original mutation from TR$^+$ to TR^s was fixed in the population by accident during the cloning procedures incidental to the isolation of *gal* K$^-$T$^-$(λ).

To test whether the cloning procedure used might have provided an inadvertent selection for TR^s variants, a reconstruction experiment was performed. The experimental procedure was identical with that used in the selection of the transduced *gal*$^+$ intermediate from which W-12 was obtained. It tests for the possibility of selection at the only step in the original isolation procedure where the mixed growth of cells with different phenotypes occurred. A small number of wild-type cells were mixed with approximately 10^8 *gal*$^-$ cells, and the mixture was plated on TTC-galactose plates. The red papillae which formed after incubation were stabbed and streaked on TTC-galactose. The *gal*$^+$ clones ob-

TABLE 1. *TR phenotype*

Strain	Designation	Origin*	Properties
Gal $K^+T^+E^+$	W-3110	EL	TR^+ wild type
Gal_2K^-	W-3102	EL	TR^+ kinaseless mutant
Gal_1T^-	W-3101	EL	TR^+ transferaseless mutant
Gal_4T^-	W-3104	EL	TR^+ transferaseless mutant
$Gal_{16}K^-E^-$	W-4221	EL	TR^+ epimeraseless mutant. Present culture also kinaseless
$Gal_2K^-(\lambda)$	W-10	JJW	TR^+ from lysogenization of W-3102 with phage λ
Gal $K^-T^-(\lambda)$	W-12	JJW	TR^s from transduction of W-3102 with λdg obtained from W-3101
$Gal_4T^-(\lambda)$	W-11	JJW	TR^+ from lysogenization of W-3104 with phage λ
Gal K^-T^-	W-3350	JJW	TR^s from W-12 by curing at high ultraviolet dosage
Gal $K^-T^-(\lambda)$	Q35A	KP	TR^s from lysogenization of W-3350 with phage λ
Gal K^-T^-(λdgal)	Q38B	KP	TR^s from W-3350 by transduction with λd*gal*$^+$
Gal K^-T^-	Q49A	KP	TR^s spontaneous segregant of Q38B
Gal K^-T^+	W6a	JJW	TR^s from transduction of W-3350 with λdg obtained from W-10, and subsequent loss of prophage
Gal K^+T^+	Q22	KP	TR^s from transduction of W-3350 with λd*gal*$^+$ and subsequent loss of prophage
Gal$^+(\lambda)$	Q33B	KP	TR^+ from lysogenization of W-3110 with phage λ
Gal$^+(\lambda^{i434})$	Q34A	KP	TR^+ from lysogenization of W-3110 with phage λ^{i434}
Gal$^+$ (re-isolated)	Q48C	KP	TR^+
Gal$^+$ (re-isolated)	Q48D	KP	TR^+

* Strain origins are designated as EL (Esther Lederberg) obtained via J. J. Weigle, JJW (J. J. Weigle), and KP (isolated in author's laboratory).

tained were purified by restreaking on the same medium and then tested for their TR^+ character. As indicated in Fig. 5, both of the strains obtained in this manner (Q48C, Q48D) were TR^+, suggesting that if any such selective factor operated, it was rather weak.

Among the strains listed, *gal*$_4$T$^-$(λ) showed some ambiguity. When overnight cultures were tested for sensitivity to transient repression by use of the simplified testing procedure, they scored as TR^s. However, although clearly not wild-type in this test, they were not typically mutant in the quantitative aspects of their response. When induction curves were performed with log-phase cultures, no transient repression was detected. It must be concluded that this strain carries some subsidiary aberration which affects the induction capacity of resting cells. There is no evidence that this aberration is related to the standard TR^s state.

Of the various strains described by this family tree, Q22, which is phenotypically *gal*$^+$ TR^s, has proved particularly useful. This strain was transduced back to galactose-fermenting ability by lysogenization with λd*gal*$^+$ with subsequent integration of the *gal*$^+$ genes and loss of λ immunity. As far as can be ascertained, this strain is now isogenic with wild-type (W-3110) except for the factor(s) responsible for the TR^s property.

Recovery from transient repression. The extended time period during which enzyme synthesis is prevented in TR^s mutants suggests that this inhibition reflects the development of the new enzyme pattern that is being established during the shift from glycerol to glucose metabolism. To test whether the eventual recovery from transient repression that occurs in glucose-growing cultures requires the formation of a new enzyme pathway, some of the conditions required for recovery have been determined.

Cultures were placed in fresh glucose medium for 60 min, which is approximately the duration of intense transient repression for β-galactosidase. Various components were omitted from the

medium, and at the end of this time the cells were tested to determine whether or not they had passed through the initial stage of transient repression and were capable of resuming enzyme induction. The criterion used was the relative ability of each culture to induce β-galactosidase with either glucose or glycerol as carbon source during the next hour. A culture still completely sensitive to transient repression will make very little, if any, enzyme during the subsequent hour of growth in glucose. In the absence of transient repression, the inducibility of β-galactosidase in glucose is 45 to 50% of that in glycerol. (The rate of enzyme induction is here defined as the increment in enzyme divided by the increment in cell mass.)

For comparison, cells previously grown both in glycerol (hence sensitive to subsequent transient repression) and in glucose (hence resistant to subsequent repression) have been used. This experiment is diagrammed schematically in Fig. 6, and the results are presented in Table 2. Cells previously grown in glycerol had partially

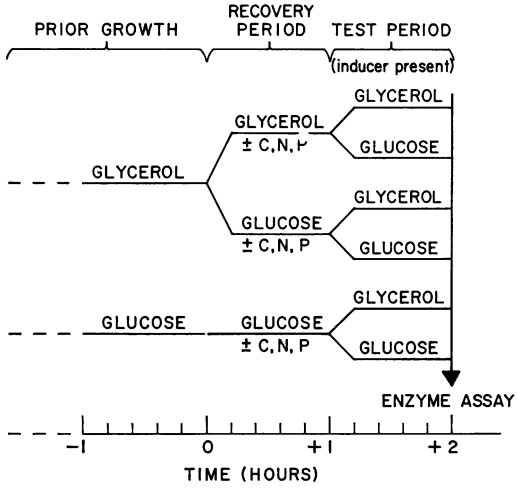

FIG. 6. *Diagrammatic representation of the recovery experiment. Starting from a very small inoculum cultures of TR^s cells (Q22) were grown to 2 × 10^8 in synthetic medium with either glycerol or glucose as carbon source (pregrowth period). They were then transferred to fresh medium buffered with 0.05 M Tris (pH 7.4) with the omission of various components of the medium. After incubation for 1 hr (escape period) the cultures were chilled and centrifuged; the cells were then washed once with carbon-free medium and divided into two parts. One part was resuspended in fresh medium containing 10^{-3} M IPTG and glycerol as carbon source. The other part was resuspended in medium containing IPTG, but with glucose as carbon source. The differential rates of β-galactosidase formation were determined for each pair of cultures during the next 60-min period.*

recovered after growth in glucose for 1 hr. This recovery was abolished if any requirement for cell growth was omitted, or if protein synthesis was blocked by chloramphenicol. It is also apparent that even glucose-grown cells that were not initially sensitive to transient repression began to become so if they were prevented from continuing protein synthesis in the presence of glucose.

Starvation-induced transient repression. The lag in inducibility which develops after starvation is probably related to transient repression, since the magnitude of the effect was dependent upon the TR character of the cells. Figure 7 shows the subsequent induction curves in glucose for TR^s and TR$^+$ cells after 3 and 8 hr of carbon starvation after an initial growth in glucose. It is apparent that even normal cells develop an induction lag after starvation, and that transient repression mutants are especially sensitive.

Model of transient repression. The behavior of cells in transient repression is most easily understood in terms of the steady state concentration of the metabolic component(s) active in catabolite repression. At any moment, the concentration of the repressor is determined by the balance struck between its rate of synthesis and rate of metabolism. These are determined by the activities of the enzymes involved and their substrate concentration dependence.

The repressor can be formed from glucose. When normal cells are shifted from glycerol to glucose as carbon source, they will increase their rate of formation of glucose-metabolizing enzymes. Eventually a new steady state concentration of repressor will be achieved. It is important to recognize that although the rate of formation of a particular enzyme may reach its new value immediately upon transfer to a new medium, the concentration of that enzyme may change only slowly as the old cytoplasm is diluted with new material. Thus the time required to reach a new steady state concentration of repressor may be quite long, the order of one or more generations, as compared with the absolute rate of metabolism of the compound.

Such transient changes in repressor concentration during the shift from glycerol to glucose metabolism are presumably responsible for the burst of tryptophanase and galactokinase formation seen after transfer to glucose. In this case, there would appear to be an initial excess of the repressor-destroying systems which is only balanced after a time by the increasing concentration of repressor forming enzyme. The burst is not seen with β-galactosidase, is small in the case of galactokinase, and is quite marked with tryptophanase. It is not known why the magnitude of this burst varies for each enzyme. As

TABLE 2. *Recovery from transient repression*[a]

Conditions during recovery period	Cells originally adapted to: Glycerol; Carbon source during recovery period: Glycerol		Cells originally adapted to: Glycerol; Carbon source during recovery period: Glucose		Cells originally adapted to: Glucose; Carbon source during recovery period: Glucose	
	Δ Cell mass[b]	Per cent[c] control	Δ Cell mass	Per cent control	Δ Cell mass	Per cent control
Complete medium	0.53	9	0.86	38	0.81	100
− Carbon	−0.02	4	−0.01	4	−0.04	43
− Phosphate	0.27	2	0.30	6	0.19	87
− Nitrogen	0.12	2	0.24	6	0.10	57
+ Chloramphenicol (10 μg/ml)	0.24	2	0.43	4	0.27	51

[a] This experiment was carried out according to the protocol described in Fig. 6.

[b] The change in cell mass of each culture measured by absorbance at 550 mμ during the 60-min recovery period.

[c] Transient repression after the recovery period was determined by enzyme formation during the test period. This is expressed as Δ β-galactosidase per Δ cell mass during growth in glucose relative to that in glycerol in the 60 min after the recovery period. The value seen in the glucose-grown culture in complete medium was 0.47. All values have been calculated relative to this number.

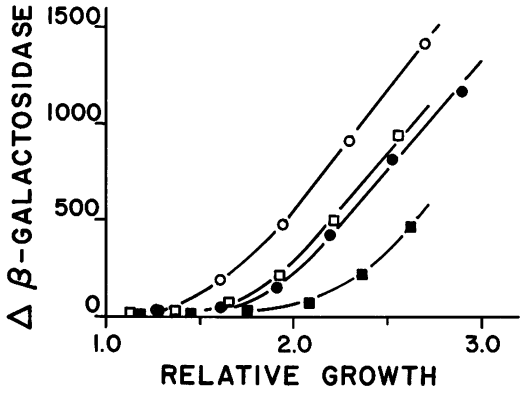

FIG. 7. *Induction lag after starvation. TR⁺ (W-3110, open symbols) and TR⁸ (Q22, closed symbols) cells were grown to 2.5 × 10⁸ in 0.1% glucose, then harvested and aerated at the same density in fresh sugar-free medium at 37 C for 3 hr (○, ●), or grown to 2.5 × 10⁸ in 0.03% glucose, which exhausted the carbon supply, and allowed to aerate an additional 8 hr (□, ■). The cultures were then harvested and resuspended at 1.5 × 10⁸ in fresh medium containing 0.25% glucose and IPTG; cell growth and β-galactosidase formation were then followed.*

mentioned below, it may depend upon the precise response of each enzyme-regulatory system to the concentration of repressor.

Viewed in terms of changes in the concentration of the catabolite repressor, transient repression mutants are most simply thought of as possessing a defect in a major repressor-destroying enzyme. Upon transfer to glucose, the repressor concentration would immediately build up, and would not begin to fall until new inducible systems for handling the repressor accumulate. Eventually a reasonable steady state level of repressor would be restored.

Such a model would explain the behavior of normal strains and TR⁸ mutants during the transition period when cells are acquiring an enzyme pattern appropriate to the new substrate. During this time the mutant cells are relatively unable to make each of the three enzymes tested. It also accounts for the bursts of enzyme synthesis seen with galactokinase and tryptophanase in the wild type, and for the necessity for protein synthesis so that cells pass through the initial stage of transient repression.

Whereas it does provide an explanation for the qualitative behavior of TR⁸ cells, such a model does not predict the quantitative differences in the responses of the three enzymes under study. However, it is unlikely that any simple model would be capable of doing so. For at least two of these enzymes, it is known that the absolute level of inducibility observed is the result of a concentration-dependent competition between inducers and repressors (Williams and Paigen, *unpublished data*). Thus the inducibility of these enzymes is influenced by the presence of other cell metabolites, which are specific to each enzyme, as well as by the component affected by the TR⁸ mutation.

Some insight into the identity of the catabolite repressor(s) has been gained by determining which carbon sources can readily generate this

compound in TR^s mutants, and which carbon sources induce the formation of enzymes capable of destroying it.

Specificity of glucose. The ability of various carbon sources to mimic glucose in producing a temporary block in enzyme induction was tested by determining the subsequent induction curves after cells pregrown in glycerol were transferred to fresh media containing a variety of possible carbon sources. These included compounds feeding the glycolytic pathway, the hexose monophosphate shunt, the Krebs cycle, and 2 carbon metabolism. The results are illustrated in Fig. 8. It is apparent that the specificity of glucose was absolute, and that no other carbon source elicited a period of transient repression.

This specificity of glucose results from its contribution to metabolism rather than from any failure on the part of glucose to participate in a reaction provided by other substrates. Thus the separate additions of lactose, galactose, gluconate, xylose, glycerol, lactate, succinate, and glycolate did not modify the period of transient repression in cells growing in glucose under conditions similar to Fig. 8. The addition of acetate considerably extended the duration of repression.

Collectively, these results suggest that the repressor is metabolically not far removed from glucose.

Susceptibility to transient repression after pregrowth in various carbon sources. Figure 9 shows the results of an experiment in which the consequent β-galactosidase induction curves in glucose were determined after pregrowth of the cells in various carbon sources. It is apparent that beyond glucose, or a glucose-generating substrate, such as lactose, only gluconate, and to some extent, xylose, were effective in reducing the period of repression. If, as suggested previously, the absence of transient repression is due to the presence of an active repressor-metabolizing system, then only glucose, and possibly gluconate, acted as exogenous inducers of this metabolic system.

Carbon source combinations. As a final test of the specificity of the effects which have been described, induction curves for β-galactosidase were determined in all possible combinations of primary and secondary carbon sources with use of glucose, fructose, xylose, glycerol, lactate, and succinate. Transient repression was only detected under the conditions which have already

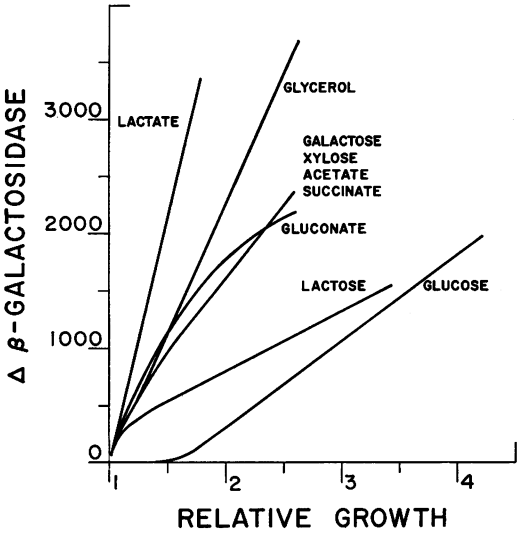

FIG. 8. *Glucose specificity in transient repression. TR^s cells (Q22) were grown to 2 × 10^8 per milliliter in glycerol medium, harvested, and resuspended separately at the same cell density in medium containing IPTG and 0.25% of each of the indicated carbon sources. Cell growth and β-galactosidase formation were then followed. The experimental points are omitted to avoid confusion.*

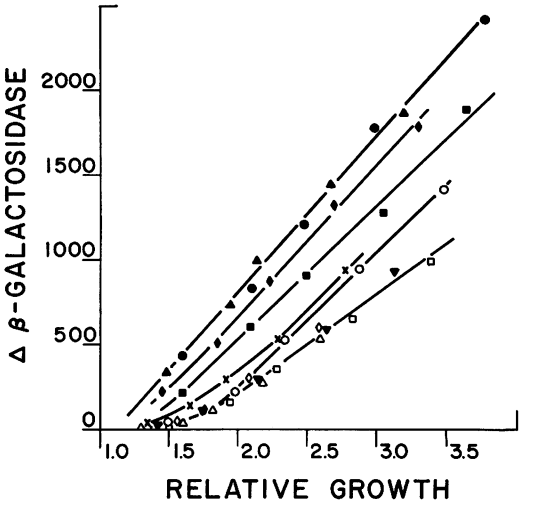

FIG. 9. *Specificity of initial carbon source in transient repression. TR^s cells (Q22) were grown to 1.5 to 2.0 × 10^8 per milliliter in separate cultures containing 0.1% of each of the carbon sources. The cells were harvested and resuspended in fresh medium containing IPTG and 0.25% glucose; growth and β-galactosidase formation were then followed. The initial carbon sources were glucose (●), lactose (▲), gluconate (◆), xylose (■), glycolate (×), glycerol (○), lactate (□), succinate (▽), galactose (△), and acetate (◇).*

been described for glucose as secondary carbon source.

DISCUSSION

The glucose effect may be considered in terms of a hypothetical low molecular weight component of the pool of intermediary metabolites. When the concentration of this component rises, either because of an increase in its rate of formation (6, 8) or a decrease in its rate of utilization (10), the synthesis of a characteristic group of enzymes is repressed. Attempts to identify this molecule(s) have so far been unsuccessful. The experiments of Neidhardt and Magasanik (9) and McFall and Mandelstam (4) suggest that different components of the pool may be responsible for the repression of the various glucose-sensitive enzymes. The common responsiveness of β-galactosidase, tryptophanase, and galactokinase to transient repression suggests that the factor disturbed in TR^s mutants is common to at least these three enzymes. No absolute identification can yet be given of the substance whose concentration is thought to regulate the inducibility of these enzymes. However, the absolute specificity of glucose in producing transient repression, and the pattern of response seen after growth in different carbon sources, suggest that the endogenous repressor is far more readily derived from glucose than from other possible substrates. This is most likely to occur if the endogenous repressor is separated from glucose by only a small number of metabolic steps.

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