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Transient Repression of the *lac* Operon

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Severe transient repression of constitutive or induced β-galactosidase synthesis occurs upon the addition of glucose to cells of *Escherichia coli* growing on glycerol, succinic acid, or lactic acid. Only mutants particularly well adapted to growth on glucose exhibit this phenomenon when transferred to a glucose-containing medium. No change in ribonucleic acid (RNA) metabolism was observed during transient repression. We could show that transient repression is pleiotropic, affecting all products of the *lac* operon. It occurs in a mutant insensitive to catabolite repression. It is established much more rapidly than catabolite repression, and is elicited by glucose analogues that are phosphorylated but not further catabolized by the cell. Thus, transient repression is not a consequence of the exclusion of inducer from the cell, does not require catabolism of the added compound, and does not involve a gross change in RNA metabolism. We conclude that transient repression is distinct from catabolite repression.

The differential rate of β-galactosidase synthesis is affected in several ways by the carbon source on which the bacteria are cultivated. A permanent severe repression of β-galactosidase synthesis results from the presence of glucose in the medium when uninduced cells are exposed to low concentrations of inducers (4, 5). Evidence has been accumulated which suggests that glucose reduces the internal concentration of inducer under these conditions, thus giving rise to incomplete induction (4, 5, 13).

A permanent weak repression of β-galactosidase synthesis occurs in cells which are induced with high concentrations of inducer (4), or which are constitutive (3, 11, 18), when glucose is present in the medium. A hypothesis accounting for this type of repression, which occurs with many catabolic enzymes, was formulated by Neidhardt and Magasanik (22), and the phenomenon was termed catabolite repression (15). Briefly stated, it postulates that catabolites which are formed from glucose accumulate in the cell and repress the formation of enzymes whose activity would augment the already large intracellular pool of these compounds. It has been shown that reduction of anabolism in constitutive cells or in cells exposed to high concentrations of inducer cells gives rise to strong repression of β-galactosidase when any carbon source is present (18). Thus, the degree of repression under these conditions appears to be determined by the concentration of catabolites.

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A severe transient repression of β-galactosidase synthesis has been observed when glucose or one of a number of other compounds is added to cultures exposed to a high concentration of inducer but previously grown on a different carbon source (2, 20, 23). Paigen (23) reported that only certain mutant strains of *Escherichia coli* exhibit this effect. Moses and Prevost (20) described a transient repression of β-galactosidase, which occurred in all strains of *E. coli* studied. Both of these groups suggested that this transient effect of glucose on β-galactosidase synthesis is an expression of catabolite repression.

Sypherd and co-workers (28, 29, 30) also observed a transient reduction of the differential rate of β-galactosidase synthesis following exposure of *E. coli* to low levels of chloramphenicol. He correlated the duration of this repression with the period of increased intracellular ribonucleic acid (RNA) concentration and suggested that RNA mediated the repression.

We have studied the effect of the addition of various compounds on the synthesis of β-galactosidase and of RNA in different strains of *E. coli*, including mutant strains constitutive for the *lac* operon and several strains that are insensitive to catabolite repression specific to the *lac* operon. The results show that transient repression of the *lac* operon is not related to an increase in RNA nor to reduction in internal inducer concentration. The repression is elicited by analogues of glucose which are not catabolized by the cell and is not relieved by a mutation that renders the cell insensitive to catabolite repression.

MATERIALS AND METHODS

Chemicals. Isopropyl-thio-β-galactoside (IPTG) and *o*-nitrophenyl-β-D-galactoside were obtained from the Mann Research Laboratories, New York, N.Y. α-Methylglucoside and 2-deoxy-D-glucose were the products of Calbiochem, Los Angeles, Calif. ^{14}C-thiomethyl-β-D-galactoside (^{14}C-TMG), ^{14}C-uracil, and ^{3}H-uracil were purchased from New England Nuclear Corp., Boston, Mass. 2,5-Diphenyloxazole (PPO) and 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) were obtained from Packard Instrument Co., La-Grange, Ill. Naphthalene and ethylene glycol were obtained from Eastman Organic Chemicals, Rochester, N.Y. Sucrose was the product of Merck and Co., Inc., Rahway, N.J. Dodecyl sodium sulfate (SDS) was obtained from Matheson, Coleman and Bell, East Rutherford, N.J.

Media. Minimal medium was made by adding to 1 liter of water 10 g of K_2HPO_4, 0.2 g of $MgCl_2$, 0.2 g of Na_2SO_4, 5.0 g of NaCl, 0.2 g of sodium citrate, 0.46 mg of $FeSO_4$, 4.4 mg of $CaCl_2$, and 0.5 mg of thiamine. The *p*H was adjusted to 7.0 with concentrated HCl. Carbon sources were added to 0.4%. $(NH_4)_2SO_4$ was present at 0.4% as nitrogen source. Streptomycin sulfate was added at a concentration of 100 μg/ml when required.

Bacterial strains. The bacterial strains used in this study are listed in Table 1. Strain LA-12 was selected for insensitivity to catabolite repression of the *lac* operon from the Hfr strain 3.000 in glucose minimal medium with *N*-acetyl lactosamine as sole source of nitrogen. Strain LA-12G, a derivative of strain LA-12, was selected for rapid growth on glucose. Strain T-9 is an F^- derivative of LA-12G obtained by the acridine orange treatment of Hirota (7). The strain was tested for the F^- character by use of the male-specific phage MS-2 as described by Revel (25). Strain T-1 is a recombinant obtained from mating strain AB311 (VHf *lac*$^-$ *str-r thr*$^-$ *leu*$^-$ *CR*$^+$) with T-9 (F^- *lac*$^+$ *str-s CR*$^-$). Strain T-5 is an independent constitutive strain obtained from strain T-1 after mutagenesis with ethyl methane sulfonate (10). All constitutive strains were shown to be of the *i*$^-$ type by mating with strain A330 as described by Loomis and Magasanik (14).

A *Salmonella typhimurium* strain carrying an F′ *lac* from *E. coli* was prepared by mating *S. typhimurium* 493H with *E. coli* K-12 strain A330 and selecting for cells able to grow on lactose minimal plates.

β-Galactosidase assay. β-Galactosidase activity was measured by the method reported by Loomis and Magasanik (11).

Thiogalactoside transacetylase assay. Thiogalactoside transacetylase activity was measured by the method reported by Alpers and Tomkins (1).

Protein synthesis. The rate of protein synthesis was estimated by determining the rate of ^{14}C-leucine uptake by the method of Nakada and Magasanik (21).

Sucrose density gradient analysis of crude extracts. Crude cell extracts were prepared by the method of Nakada and Magasanik (21) with one modification. After the cells were ground with alumina, the pellet was extracted with a small volume of buffer [5 mM tris(hydroxymethyl)aminomethane (Tris; *p*H 7.25)-0.1 mM magnesium acetate], deoxyribonuclease (5 μg/ml) was added, the extract was freed of alumina and debris by two centrifugations at 20,000 × *g* for 20 min, and then SDS was added to 0.5% final concentration; 1.0 ml of this extract was layered over 24 ml of a linear sucrose gradient, 5 to 20% sucrose in SDS buffer [0.01 M Tris (*p*H 7.4), 0.1 M NaCl, 0.001 M ethylenediaminetetraacetic acid, 0.5% SDS], and subjected to centrifugation at 25,000 rev/min for 10.5

TABLE 1. *Bacterial strains*

Strain	Pertinent genotype[a]	Source or reference[b]
Escherichia coli K-12		
3.000	Hfr H *lac*$^+$ (*i*$^+$*z*$^+$*y*$^+$) *CR*$^+$ *str-s*	a
C600	F^- *lac*$^-$ (*i*$^+$*z*$^+$*y*$^-$) *CR*$^+$ *thr*$^-$ *leu*$^-$ *str-r*	a
A330	*lac*$^-$ (*i*$^+$*z*$^+$*y*$^-$)/F′ *lac* (*i*$^+$*z*$^+$*y*$^+$) *str-s pro*$^-$ *his*$^-$ *arg*$^-$	a
AB311	VHf *lac*$^-$ (*i*$^+$*z*$^+$*y*$^-$) *CR*$^+$ *str-r thr*$^-$ *leu*$^-$	a
3.300	Hfr H *lac*$^+$ (*i*$^-$*z*$^+$*y*$^+$) *CR*$^+$ *str-r*	a
2.000 o[c]	F^- *lac*$^+$ (*i*$^+$*o*^{c}*z*$^+$*y*$^+$) *CR*$^+$ *str-r*	a
LA-12	F^+ *lac*$^+$ (*i*$^+$*z*$^+$*y*$^+$) *CR*$^-$ *str-s*	b
LA-12G	F^+ *lac*$^+$ (*i*$^+$*z*$^+$*y*$^+$) *CR*$^-$ *str-s*	b
T-1	F^- *lac*$^+$ (*i*$^+$*z*$^+$*y*$^+$) *CR*$^-$ *str-r*	
T-5	F^- *lac*$^+$ (*i*$^-$*z*$^+$*y*$^+$) *CR*$^-$ *str-r*	
T-9	F^- *lac*$^+$ (*i*$^+$*z*$^+$*y*$^+$) *CR*$^-$ *str-s*	
Aerobacter aerogenes 35	*lac*$^+$ (*i*$^+$*z*$^+$*y*$^+$) *CR*$^+$	c
Salmonella typhimurium 493H		c
S. typhimurium 493H	F′ *lac*$^+$ (*i*$^+$*z*$^+$*y*$^+$)	d

[a] The sex, *lac*, and streptomycin symbols are identical to those in Loomis and Magasanik (12). *CR* refers to catabolite sensitivity of the *lac* operon; *thr*$^-$, *leu*$^-$, *pro*$^-$, *his*$^-$, and *arg*$^-$ refer, respectively, to the inability to synthesize threonine, leucine, proline, histidine, and arginine. Where these are indicated, the compound was supplied in the growth medium at a concentration of 20 μg/ml.

[b] (a) S. E. Luria; (b) Loomis and Magasanik (12); (c) Boris Magasanik; (d) Rodney Wishnow. All other strains were isolated during the course of this work.

hr at 15 C. The bottom of the centrifugation tube was pierced with a hypodermic needle, and fractions of 40 drops each were collected through a Gilford recording spectrophotometer (model 2000), which plotted the optical density at 260 mμ. Each fraction received 0.05 ml of 1% yeast RNA and then was precipitated with 1.2 ml of cold trichloroacetic acid. The precipitates were collected on membrane filters (Millipore Corp., Bedford, Mass.; 0.45 μ) and washed with 5 volumes of cold 5% trichloroacetic acid. The filters were placed in polyethylene vials (Packard Instrument Co., Inc., LaGrange, Ill.) and dried. The vials were then filled with 20 ml of Brays solution (naphthalene, 60 g; PPO, 4 g; POPOP, 0.2 g; methanol, 100 ml; ethylene glycol, 20 ml; dioxane, 834 ml), and counted in a liquid scintillation counter (model 725; Nuclear-Chicago Corp., Des Plaines, Ill.).

RESULTS

Transient repression and catabolite repression. Paigen (23) reported that certain mutants of *E. coli* K-12 show a strong (approximately 90%) transient repression of β-galactosidase synthesis when transferred from a medium containing glycerol as the major source of carbon to a medium containing glucose as the major source of carbon; the parent strain did not exhibit this effect. On the other hand, Moses and Prevost (20) reported that seven unrelated strains of *E. coli* exhibit transient repression of β-galactosidase when glucose is *added* to the glycerol-containing medium.

To compare the effects of these two alterations of the environment on the same strain of *E. coli* K-12, we grew strain 3.000 in a medium containing glycerol as the only source of carbon, collected the cells, and placed them in media containing glycerol alone, glucose alone, or a mixture of glycerol and glucose. IPTG was added, and growth and β-galactosidase formation were measured. All cultures grew promptly and, in the case of the cultures transferred to glucose-containing medium, at the increased rate characteristic of the new medium. Enzyme activities of the cultures were determined and were plotted against the increases in cell mass (Fig. 1a). Cells placed in the medium containing glucose only produced β-galactosidase at a differential rate approximately one-half that observed in the cells growing in the medium containing glycerol only. This reduction in rate is the characteristic result of catabolite repression. The cells placed in the medium containing glycerol as well as glucose produced the enzyme initially at an extremely low rate; only after the cell mass had increased by about one-half did the rate of enzyme production equal that of the culture growing on glucose alone. In a control experiment, the cells also did not experience transient repression when transferred into a medium containing glycerol alone or glucose alone at the concentration finally produced by the presence of both sugars when

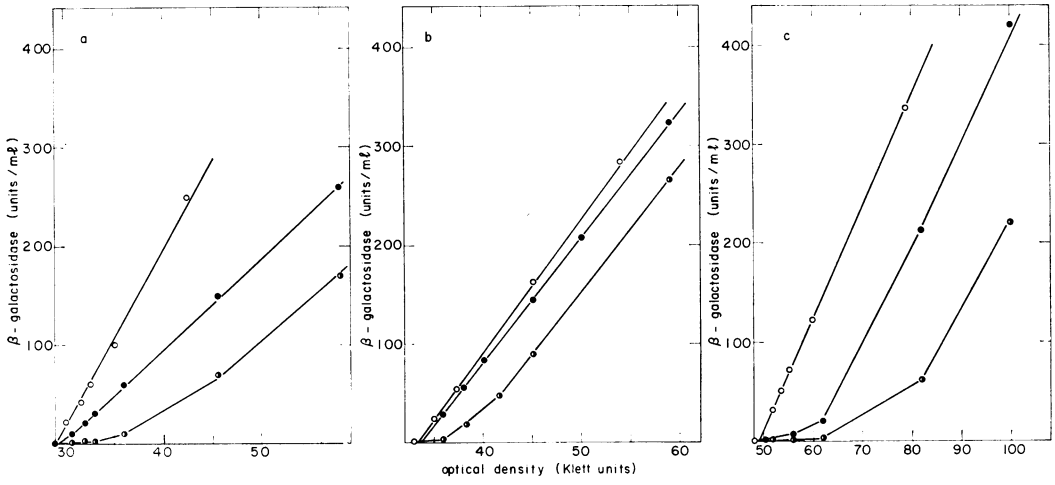

FIG. 1. *Transient repression of β-galactosidase. Cultures of Escherichia coli K-12 strains 3.000, LA-12, and LA-12G in the exponential phase of growth were membrane-filtered, washed, and suspended in minimal salts medium without a carbon source (an optical density of 30 Klett units is about 10^8 viable bacteria/ml); each suspension was immediately divided into three parts, which received 0.4% glycerol, 0.4% glucose, or 0.4% glycerol and 0.4% glucose. IPTG (10^{-3} M) was added to each culture, and samples were taken from each culture for assay of β-galactosidase activity.* (○) *Culture containing only 0.4% glycerol;* (●) *culture containing only 0.4% glucose;* (◑) *culture containing both 0.4% glycerol and 0.4% glucose. (a) Strain 3.000; (b) strain LA-12; (c) strain LA-12G. The mass doubling times on glycerol were: 3.000, 90 min; LA-12, 100 min; LA-12G, 90 min. The mass doubling times on glucose were: 3.000, 65 min; LA-12, 85 min; LA-12G, 60 min.*

transient repression was elicited. These results show that, in glycerol-grown wild-type *E. coli* K-12, both glycerol and glucose are required for transient repression.

We examined an additional six strains derived from *E. coli* K-12. All exhibited transient repression of β-galactosidase when glucose together with the inducer was added to a culture growing in a glycerol-containing medium. In addition, we found transient repression of the enzyme under these conditions in *Aerobacter aerogenes* and in a strain of *S. typhimurium* carrying an F′ *lac* episome derived from *E. coli* K-12. (The experiment on *S. typhimurium* was done by Rodney Wishnow.)

To assess the relation of transient repression to catabolite repression, we used a mutant, strain LA-12, which has become specifically insensitive to catabolite repression (CR^-) of β-galactosidase (12). The results (Fig. 1b) clearly show this insensitivity. The mutant produced β-galactosidase at essentially the same rate in medium containing either glycerol or glucose as the sole source of carbon. On the other hand, the mutant was still subject to transient repression; when placed after growth on glycerol in a medium containing a mixture of glucose and glycerol, an almost complete repression of β-galactosidase synthesis occurred initially. The cells recovered from this repression and produced β-galactosidase eventually at the same differential rate in the medium containing glycerol alone, glucose alone, or glycerol and glucose together (Fig. 1b). Increasing the concentration of only glycerol or glucose to that produced by glycerol and glucose together did not elicit transient repression.

A mutant of strain LA-12 had been previously selected for its ability to grow more rapidly on glucose than its parent (12). We examined the susceptibility of this strain, LA-12G, to repression by glucose in the same manner as that of strains 3.000 and LA-12. In cells placed after growth on glycerol into a medium containing only glucose, β-galactosidase synthesis was initially strongly repressed (Fig. 1c). This period of repression was somewhat shorter than the period of repression experienced by the cells placed in the medium containing both glucose and glycerol. In this respect, strain LA-12G resembles the mutant described by Paigen (23) as subject to transient repression by glucose. However, eventually the cultures growing on glucose alone or on the mixture of glucose and glycerol produced β-galactosidase at the same differential rate as did the culture growing on glycerol alone. No repression of β-galactosidase synthesis was elicited by glycerol in this strain even when the concentration was increased above that produced by glycerol and glucose together. It is apparent that the mutation leading to better growth on glucose has enhanced the susceptibility of the organism to transient repression, but has not restored its susceptibility to catabolite repression.

It is of interest that cultivation on glycerol is not essential for transient repression. The addition of glucose to a culture of strain LA-12G growing on either glycerol or lactate resulted in transient repression (Table 2). On the other hand, the addition of glycerol to cultures of this organism growing on lactate or glucose did not interfere with the production of the enzyme.

We could show that transient repression is not a consequence of the interference by glucose with the uptake of the inducer in uninduced cells. We found, in agreement with an earlier report (20), that the constitutive synthesis of β-galactosidase is subject to transient repression. The addition of glucose to cells of an i^- strain, 3.300, or an O^c strain, 2.000, growing on glycerol produced a strong transient reduction in the differential rate of enzyme synthesis (Fig. 2). After recovery, β-galactosidase was produced in the presence of glucose and glycerol at approximately one-half the rate found when glycerol alone was present, that is, at the rate characteristic of catabolite repression. In cells which were i^- as well as CR^-, the transient repression of enzyme formation was also evident; however, in this case, following recovery, enzyme was produced at the same rate in the absence or presence of glucose.

Role of glucose in transient repression. Sypherd and Strauss (29, 30) and Sypherd and DeMoss (28) observed a transient repression of β-galactosidase in cells treated with chloramphenicol at

TABLE 2. *Transient repression in strain LA-12G*[a]

Pregrown on	Carbon source added at time of induction	Rate of β-galactosidase synthesis during first 20 min after induction
Glycerol	Glucose	10
Lactate	Glucose	15
Lactate	Glycerol	100
Glucose	Glycerol	100

[a] The various carbon sources were added at 0.4% to cultures of strain LA-12G growing in minimal medium on glycerol, DL-lactate, or glucose; 10^{-3} M IPTG was also added at this time. The values are relative to the rate in the absence of added carbon source, which is taken as 100. In all cultures receiving additions, the rate of β-galactosidase synthesis increased after the first 20 min.

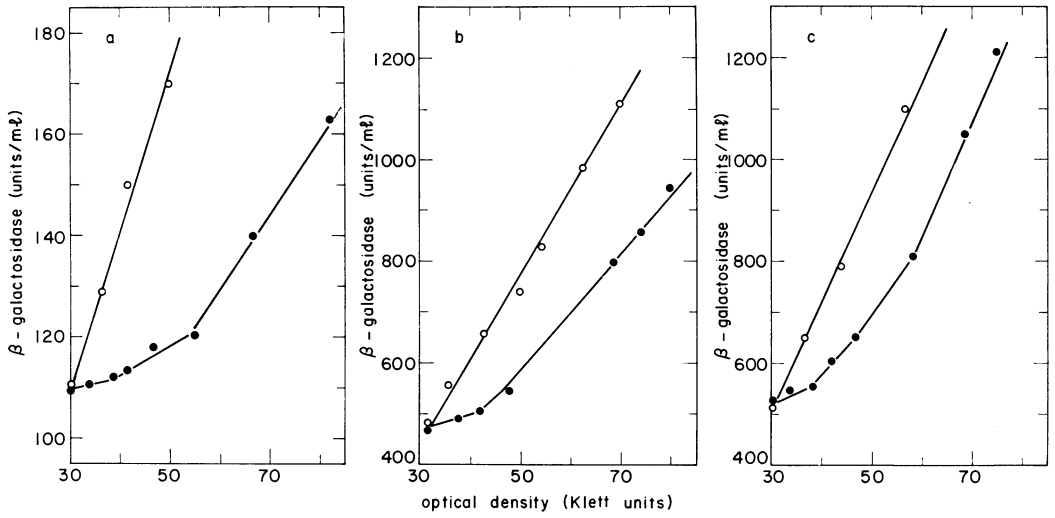

FIG. 2. *Transient repression in lac constitutive strains of Escherichia coli K-12. Cultures of E. coli K-12 strains which show constitutive synthesis of the lac operon were grown into exponential phase on minimal salts medium containing glycerol as the sole carbon source; the cultures were then divided and glucose (0.4%) was added to one portion. Samples were taken from each culture for assay of β-galactosidase activity; growth was followed by optical density.* (○) *Culture containing only glycerol as a carbon source;* (●) *culture containing both glycerol and glucose as carbon sources.* (*a*) *Strain 2.000 O^c;* (*b*) *strain 3.300;* (*c*) *strain T-5.*

a level insufficient to arrest growth completely. This treatment reduces the rate of protein synthesis more drastically than the rate of RNA synthesis. Sypherd and Strauss (29) suggested that the RNA produced in excess of protein might be responsible for the repression of the enzyme. We investigated, therefore, whether the metabolism of glucose by cells of strain T-1 growing on glycerol causes a preferential increase in the rate of incorporation of exogenous uracil into RNA. The rate of growth increased promptly upon additon of glucose; on the other hand, RNA synthesis proceeded at the same rate in the culture containing glycerol only and in the culture containing both glycerol and glucose throughout the period of transient repression (Fig. 3). The same results were obtained when a uracil-requiring mutant was used. We may therefore conclude that the addition of glucose does not cause a preferential increase in the rate of RNA synthesis.

We obtained additional evidence against a role for RNA synthesis in transient repression by demonstrating that the repression can occur without any net increase in RNA. It has been shown previously that CR^- strains, in contrast to CR^+ strains, can produce β-galactosidase during nitrogen starvation in the presence of a utilizable source of carbon (12). Thus, glucose-grown cells of strain LA-12G produce the enzyme at the same rate in ammonia-free media with or without glucose, and glycerol-grown cells of LA-12G produce the enzyme at the same rate in ammonia-free media with or without glycerol. A similar experiment was carried out with a derivative of LA-12, strain T-1, except that in this case the glycerol-grown cells were induced in ammonia-free media containing either glycerol or glucose and glycerol (Fig. 4). Enzyme was produced readily in the medium containing only glycerol, but not in the medium containing glucose as well as glycerol. It is apparent that glucose exerts transient repression even when net RNA synthesis is prevented by the absence of an available source of nitrogen.

The breakdown and resynthesis of the many species of unstable RNA proceeds during nitrogen starvation (19, 27). We considered the possibility that the metabolism of glucose could alter the distribution of the different RNA species. Such a change might be revealed by labeling the RNA produced under nitrogen starvation with radioactive uracil and analyzing the cell extracts on a sucrose gradient. We assume that the exogenous uracil, having entered the intracellular pool, would be used with the same efficiency for the formation of all species of RNA. We suspended glycerol-grown cells of strain T-1 in an ammonia-free medium containing glycerol and divided the culture into two flasks. After 5 min of incubation we added ^{14}C-uracil to one flask, and ^{3}H-uracil and glucose to the other. Exactly 2

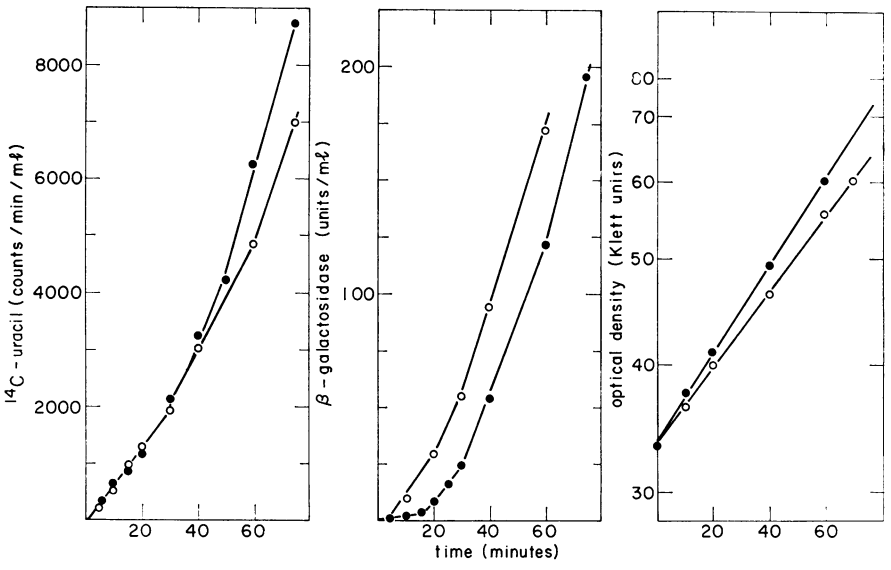

FIG. 3. *RNA synthesis in Escherichia coli during transient repression. A culture of exponential-phase E. coli T-1 growing in minimal medium containing glycerol was divided into two parts. One part received 0.4% glucose; the other part served as a control. Both parts received 10^{-3} M IPTG and ^{14}C-uracil (0.1 μc and 12 μg per ml) at time zero. Samples were taken for assay of enzyme activity and incorporation of ^{14}C-uracil into RNA. Growth was followed by optical density. (●) Culture containing both glycerol and glucose; (○) culture containing only glycerol.*

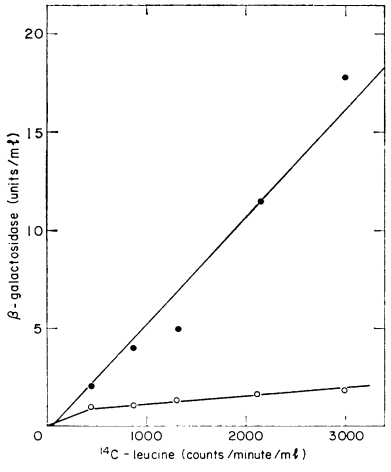

FIG. 4. *Transient repression of β-galactosidase in nitrogen-free medium. Cells of a culture of strain T-1 growing exponentially in glycerol minimal medium were collected and washed on membrane filters. The cells were resuspended in nitrogen-free minimal medium. Half the culture received 0.4% glycerol (●); the other half received both 0.4% glycerol and 0.4% glucose (○). IPTG (10^{-3} M) was added to both cultures at zero-time. Samples were taken for assay of β-galactosidase activity and incorporation of ^{14}C-leucine (90 min).*

min later, the contents of both flasks were poured into the same beaker, containing crushed frozen Tris buffer. After thawing, the cells were collected and disrupted, and the extract was analyzed on a sucrose gradient for absorption at 260 mμ and radioactivity due to the two isotopes (Fig. 5). In both cases, most of the radioactivity was found in an area between the peaks of ultraviolet absorption characteristic of the 30*S* ribosomal particle and the 4*S* RNA. The sedimentation patterns of the radioactive macromolecules produced on glycerol alone or on glycerol plus glucose as a carbon source were remarkably similar. Essentially the same result, similar patterns in the two cultures, was obtained when the nitrogen-starved cells were exposed to radioactive uracil for 60 min, although in this case a far greater proportion of the label appeared in the area of 16*S* and 28*S* RNA. The cells treated with glucose incorporated somewhat more radioactivity into macromolecules than did the cells exposed only to glycerol. However, this may be merely a reflection of a more efficient conversion of exogenous uracil to intracellular uridine triphosphate. Since glucose produces no significant difference in the sedimentation pattern, this experiment does not support the idea

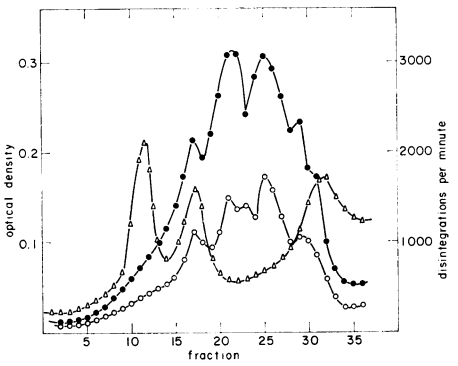

FIG. 5. *Sedimentation pattern of RNA labeled with ^{3}H-uracil and ^{14}C-uracil in a nitrogen-free medium. Cells were exposed for 2 min to either ^{3}H-uracil (0.5 μc and 1.85 μg per ml) in a medium containing both glucose and glycerol but no nitrogen or to ^{14}C-uracil (0.5 μc and 1.85 μg per ml) in a medium containing only glycerol and not nitrogen. (△) Absorbancy at 260 mμ; (○) ^{14}C disintegrations in RNA; (●) ^{3}H disintegrations in RNA.*

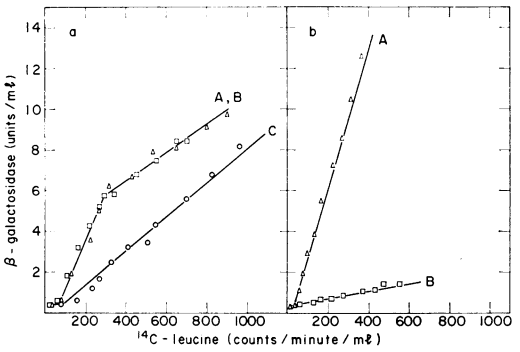

FIG. 6. *Kinetics of catabolite repression and transient repression. Exponential-phase cells of the CR^{+} Escherichia coli strain 3.000, which had been grown on either glucose or glycerol, were filtered and suspended in a carbon-free medium for 5 min before IPTG (10^{-3} M), ^{14}C-leucine (0.1 μc and 10 μg per ml), and either the carbon source (0.4%) to which they had been preconditioned or both glucose (0.4%) and glycerol (0.4%) were added. As a control, an additional sample of the cells conditioned to glucose was filtered, suspended directly in glucose-containing medium, and then treated with ^{14}C-leucine and IPTG 5 min later. Samples were taken from each culture for assay of β-galactosidase activity and determination of radioactivity incorporated into protein. (a) Glucose-conditioned cells. Curve A (△), culture which received glucose after starvation; curve B (□), culture which received both glucose and glycerol; curve C (○), control culture, not starved. (b) Glycerol-conditioned cells. Curve A (△), culture which received glycerol after starvation; curve B (□), culture which received both glucose and glycerol after starvation.*

that transient repression is the result of an alteration of RNA synthesis.

We decided to examine next whether the accumulation of catabolites derived from glucose is responsible for transient repression. It has been shown previously that glucose-grown cells can be made transiently insensitive to catabolite repression of β-galactosidase (21). This phenomenon is illustrated in Fig. 6a. Glucose-grown cells of the CR^{+} strain 3.000 were incubated for 5 min in a medium lacking any source of energy; the inducer, ^{14}C-leucine, and glucose or both glucose and glycerol were then added. These cells (curves A and B) produced the enzyme initially at a rate higher than that of glucose-grown cells, which had not experienced the period of starvation for a source of energy (curve C). It appears that glucose cannot replenish the catabolite pools of the starved cells sufficiently fast to prevent this escape from repression. When these starved cells were induced in the presence of glycerol alone, the enzyme was made throughout the experiment at the unrepressed rate which was observed only initially when glucose was present after the starvation. Thus, it appears that considerable protein synthesis occurs before the metabolism of glucose has generated a pool of catabolites large enough to cause catabolite repression.

In contrast, no measurable delay occurred in the establishment of transient repression when glucose was allowed to act on glycerol-grown cells that had experienced a similar 5-min period of energy starvation. These cells produced the enzyme rapidly when induced in a medium containing only glycerol (curve A), but not in a medium containing glucose as well as glycerol (curve B). In the latter case, a severe repression was established before any measurable protein synthesis occurred. After about 15 min, the differential rate of β-galactosidase synthesis began to increase until, after 30 min, it exhibited only the weak repression characteristic of catabolite repression. In a control experiment, no severe transient repression occurred when these energy-starved cells were induced in the presence of glucose alone.

These results suggest that the repression of β-galactosidase synthesis which results from the additon of glucose to cells growing on glycerol is not a consequence of an augmentation of the pools of catabolites by the degradation of glucose. The effect rather appears to be directly attributable to the initial intracellular accumulation of glucose. In that case, analogues of glucose which cannot be catabolized should exert

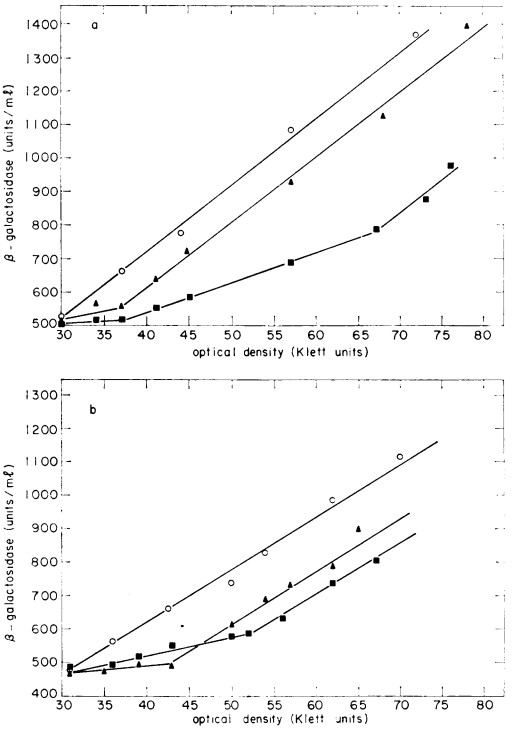

FIG. 7. *Transient repression by glucose analogues. An exponential-phase culture of CR⁻ strain T-5 or CR⁺ strain 3.300 was split into four parts. One part served as the control. Each of the other three parts received 0.4% glucose, 0.4% α-methyl glucoside, or 0.4% 2-deoxyglucose at time zero. Growth was followed by optical density. Samples were taken from each culture for assay of β-galactosidase activity. (a) Strain T-5; (b) strain 3.300.* (○) *Control culture containing only glycerol;* (▲) *culture containing both glycerol and α-methylglucose;* (■) *culture containing both glycerol and 2-deoxyglucose. Data from cultures which received glucose are shown in Fig. 2.*

transient repression, but not catabolite repression. That this is indeed true is shown by the experiments illustrated in Fig. 7. Cells of the constitutive, catabolite-sensitive strain 3.000 ($i^-z^+y^+$ CR^+) and of the constitutive catabolite-insensitive strain T-5 ($i^-z^+y^+$ CR^-) were grown on glycerol and treated with α-methyl glucoside and 2-deoxyglucose. Since these compounds do not support any growth of the cells, they must not serve as sources of energy and carbon or contribute to the common catabolite pool. Their additon to the medium already containing glycerol did not alter the growth rate of the cells for the 100 min during which enzyme synthesis was observed. The results show clearly the transient repression that ensued upon addition of the glucose analogues. As expected, these compounds do not serve as sources of repressing catabolites; after recovery from transient repression, both the catabolite-sensitive and the catabolite-insensitive strain produced the enzyme at the same rate in the absence or presence of the analogues.

These results would seem to indicate that glucose does not elicit transient repression by provoking a gross change in RNA metabolism nor through its own catabolism.

Coordinate control by transient repression. It has been shown that the three components of the *lac* operon, β-galactosidase, β-galactoside permease, and thiogalactoside transacetylase respond in coordinate fashion to induction (8). We tested the response of the three activities to transient repression by glucose. The synthesis of β-galactosidase and the ability to maintain a high intracellular concentration of TMG were compared in a culture of strain LA-12G. The cells were grown on glycerol in the presence of 10^{-3} M IPTG overnight. They were then suspended in a medium containing glycerol, as source of carbon, and 10^{-3} M ^{14}C-TMG, and were permitted to grow for a period of 2 hr. Glucose was then added to one-half of the culture, and samples were withdrawn at intervals over a period of 80 min for the assay of β-galactosidase and of intracellular ^{14}C-TMG. In the absence of glucose, both the total amount of enzyme and the total amount of ^{14}C-TMG bound to cells continued to increase; in the presence of glucose, there was a transient arrest in the increase of both enzyme and cell-bound ^{14}C-TMG (Fig. 8). This is the result expected if glucose transiently arrested the synthesis of both enzyme and permease, but without interfering with the activity of the pre-existing permease.

Unfortunately, the assay of cell bound ^{14}C-TMG is not sufficiently accurate to prove that β-galactosidase and β-galactosidase permease respond coordinately to transient repression. We demonstrated a coordinate response more convincingly with the use of β-galactosidase and thiogalactoside transacetylase. In glycerol-grown cells of strain LA-12G, both enzymes exhibited the same kinetics of transient repression by glucose (Fig. 9). (The thiogalactoside transacetylase assays were done by Allen E. Silverstone.) After recovery from transient repression, each enzyme was produced in the medium containing glucose and glycerol at the same rate as in the medium containing glycerol only. It is evident that, in strain LA-12G, both enzymes are insensitive to catabolite repression.

In summary, the results show that, like the repression of the *lac* operon mediated by the *i*-gene product, transient repression also affects coordinately all proteins of the *lac* operon.

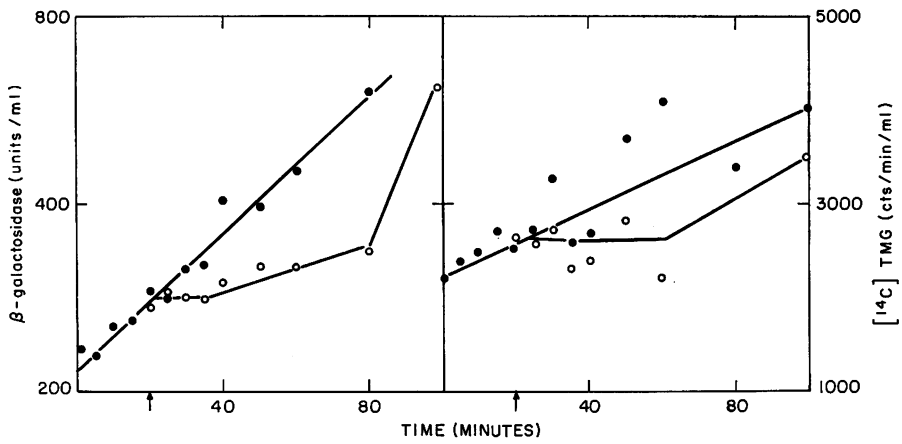

FIG. 8. *Transient repression and TMG content of fully induced cells. (a) Samples were taken for determination of β-galactosidase activity from a culture of strain LA-12G ($i^+z^+y^+$ CR^-) growing exponentially in glycerol minimal medium containing 10^{-3} M ^{14}C-TMG. (b) Samples were also taken for determination of ^{14}C-TMG content. At 20 min, glucose was added to half the culture. (●) Glycerol culture; (○) culture to which glucose was added.*

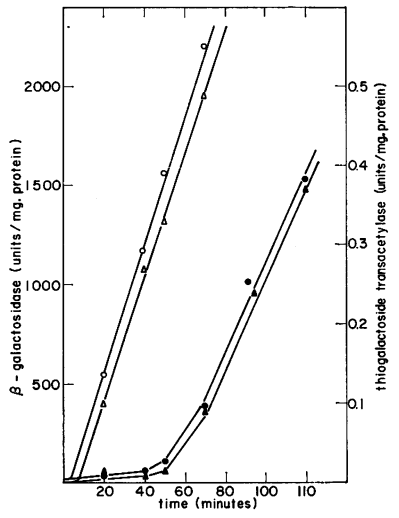

FIG. 9. *Transient repression of thiogalactoside transacetylase. A culture of Escherichia coli LA-12G in the exponential phase of growth (optical density 35 Klett units) on minimal salts medium containing glycerol was divided into two parts. One part received IPTG (10^{-3} M) and glucose (0.4%); the other part received only IPTG. Samples were taken for assay of β-galactosidase (○, ●) and transacetylase activity (△, ▲). Open symbols, glycerol culture; closed symbols, glucose culture.*

DISCUSSION

These results show that transient repression is a general phenomenon occurring in all strains of *E. coli* tested, as well as in strains of *A. aerogenes* and *S. typhimurium*, if glucose or one of several other compounds is added to cultures of cells still in contact with the carbon source on which they were pregrown. If, however, the cells are transferred from the carbon source on which they were pregrown to a medium containing only glucose, then only strains which appear particularly well adapted to growth on glucose will display transient repression.

Unlike the severe repression observed in uninduced cells when low levels of inducer are used with glucose (4, 5, 13), the transient repression described here is not the result of a reduction in the internal concentration of inducer, since the effect is seen in strains constitutive for β-galactosidase whether or not inducer is present.

Sypherd and co-workers (28–30) observed a transient repression of β-galactosidase after the addition of chloramphenicol and correlated this with the net increase in RNA within the cells. Our observations would seem to rule out RNA as an effector of the transient repression studied here.

Finally, we have presented evidence indicating that transient repression is not an expression of extreme catabolite repression. We have shown that transient repression is independent of a mutation in the *CR* gene which allows CR^- strains adapted to glucose to synthesize β-galactosidase in the presence of glucose even when anabolism is limited by nitrogen starvation (12). It does not seem likely that the addition of glucose to growing cells would result in a larger catabolite pool than that established in nitrogen-starved cells incubated in the presence of glucose.

Addition of α-methyl glucoside or 2-deoxyglucose to *E. coli* K-12 cells growing on glycerol

results in a period of severe transient repression, but these compounds fail to repress β-galactosidase synthesis after this period. Although these compounds are apparently transported and phosphorylated, they are not metabolized further (6, 26, 31); presumably, they do not enter the common catabolite pool involved in catabolite repression (15). Therefore, it seems probable that the phosphorylated compound itself is the effector of transient repression. This idea is supported by the recent work of Prevost and Moses (24), who have shown that the pool size of glucose-6-phosphate, 6-phosphogluconate, fructose-1,6-diphosphate, and reduced nicotinamide adenine dinucleotide phosphate increases when glucose is added to cells growing on glycerol and then begins to decrease as the cells emerge from transient repression. Escape from transient repression must then occur as the cell adjusts to the new intracellular level of phosphorylated compounds or appropriately decreases the concentration of these compounds.

We have shown that transient repression has a pleiotropic effect on all products of the *lac* operon. It appears that this control is effective as soon as the new compound enters the cell. In contrast, about 8 min of protein synthesis at 37 C elapses before catabolite repression due to glucose reduces the differential rate to about 50%. Thus, it appears that the two types of repression, catabolite repression and transient repression, are quite distinct.

In summary, we have shown that transient repression is a general phenomenon which affects the differential rate of β-galactosidase immediately upon the addition of many compounds to cells growing on another carbon source. Transient repression does not result from a reduction in the internal level of inducer and is apparently elicited by a mechanism distinct from that which mediates catabolite repression.

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