JOURNAL OF BACTERIOLOGY, July, 1966
Vol. 92, No. 1

Nature of the Effector of Catabolite Repression of β-Galactosidase in *Escherichia coli*

WILLIAM F. LOOMIS, JR.,[1] AND BORIS MAGASANIK

Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts

Received for publication 1 April 1966

ABSTRACT

LOOMIS, WILLIAM F., JR. (Massachusetts Institute of Technology, Cambridge, Mass.), AND BORIS MAGASANIK. Nature of the effector of catabolite repression of β-galactosidase in *Escherichia coli*. J. Bacteriol. **92**:170–177. 1966.—Many carbon sources were found to give rise to catabolite repression of β-galactosidase in a mutant strain of *Escherichia coli* lacking hexose phosphate isomerase activity. Compounds containing glucose or galactose cannot be formed from several of these carbon sources in this mutant strain, and, therefore, appear not to be required for catabolite repression of β-galactosidase. Glucose was observed to elicit catabolite repression of β-galactosidase in another mutant strain under conditions in which the formation of compounds of the citric acid cycle is inhibited. If catabolite repression of the *lac* operon is mediated by a single compound, it appears that the compound is related to the pentoses and trioses of intermediary metabolism. The repression of β-galactosidase by galactose in galactokinase negative strains was shown to be independent of the gene, *CR*, which determines catabolite sensitivity of the *lac* operon, and to be dependent on a functional *i* gene.

The theory of catabolite repression (18) postulates that the degree of repression of an operon depends on the concentration of one or more specific catabolites which can be formed from many different carbon sources, including the substrate of the repressible enzyme. Many biochemical steps in catabolism are reversible. It is, therefore, very difficult to determine which specific compound is involved in the control of an enzyme by analyzing the repression elicited by different carbon sources. However, the relative facility with which a carbon source gives rise to the catabolite can be determined. This approach has been used for study of the control of β-galactosidase (8, 17). Glucose was found to be a better source of the compound related to the *lac* operon than is glycerol. Pyruvate was found to be a poor source of the catabolite related to control of β-galactoside. However, it was shown that pyruvate is a good source of the catabolite related to control of tryptophanase; although the specific compound involved in the control of either of these genes is unknown, it appears that the two genes are controlled by different catabolites.

McFall and Mandelstam (17) suggested that a high concentration of galactose, galactose-1-phosphate, or uridine diphosphate (UDP) galactose per se gives rise to catabolite repression of β-galactosidase. They found that the presence of galactose in the medium would repress β-galactosidase in galactose-negative mutants blocked in galactokinase, galactose transferase, or UDP galactose epimerase. Since the conversion of galactose to glucose is impaired in these strains, galactose compounds per se appeared to exert repression. McFall and Mandelstam (17) showed that glucose gives rise to the same degree of catabolite repression of β-galactosidase in galactokinase- and galactose transferase-negative mutants as in wild-type strains. They concluded that UDP galactose alone could give rise to catabolite repression. When glucose was present in the medium of a UDP galactose epimerase-negative mutant, catabolite repression of β-galactosidase could be observed, but appeared weaker than in the wild-type strain. They concluded that only the galactose compounds are involved in catabolite repression of β-galactosidase and that the glucose-elicited repression observed in the epimerase-negative mutant was the result of residual epimerase activity.

Our studies with a mutant lacking hexose phos-

[1] Present address: Department of Biology, Brandeis University, Waltham, Mass.

phate isomerase suggest that compounds other than those related to galactose can rise to catabolite repression of β-galactosidase.

The characterization of a gene, *CR*, which determines catabolite sensitivity specific for the *lac* operon, and the isolation of a CR^- mutant strain (15), allowed us to determine whether the repression of β-galactosidase by galactose in galactose-negative mutants is dependent on a functional *CR* gene. The results suggest that galactose can inhibit induction of the *lac* operon independently of catabolite repression.

MATERIALS AND METHODS

Chemicals. Isopropyl-thio-β-D-galactoside (IPTG) and *o*-nitrophenyl-β-D-galactoside (ONPG) were obtained from Mann Research Laboratories, New York, N.Y.

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, traces of $FeSO_4$ and $CaCl_2$, 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. Individual amino acids were added to 20 μg/ml when required.

EMB-galactose medium was prepared by adding to 1 liter of water 8 g of tryptone, 5 g of NaCl, 1 g of yeast extract, 15 g of agar, 400 mg of eosin, 64 mg of methylene blue, 2 g of K_2HPO_4, and 10 g of galactose. Tryptone medium was made by adding to 1 liter of water 10 g of tryptone, 5 g of NaCl, and 5 g of yeast extract. The *p*H was adjusted to 7.0 with 5 N NaOH.

Phage and bacterial strains. Transducing phage P1kc was obtained from the collection of S. E. Luria. The bacterial strains used in this study are listed in Table 1.

β-Galactosidase assay. β-Galactosidase activity was measured by the method reported by Loomis and Magasanik (14).

Hexose phosphate isomerase. The specific activity of hexose phosphate isomerase was determined in cell extracts by observing the rate of reduction of nicotinamide adenine dinucleotide phosphate (NADP) at 37 C on a Beckman DU spectrophotometer attached to a Gilford absorbance recorder at 340 mμ. Washed, concentrated bacterial cultures in 0.04 M glycylglycine-HCl buffer (*p*H 7.4) were disrupted in an ultrasonic oscillator (Measuring & Scientific Equipment, Ltd., London, England). The extracts were freed from debris by centrifugation. To 2.6 ml of 0.04 M glycylglycine buffer (*p*H 7.4) was added 0.1 ml of a solution 6×10^{-1} M $MgCl_2$, 0.1 ml of a solution 4.5×10^{-3} M NADP, 0.1 ml of extract, and 0.1 ml of a solution 2×10^{-2} M fructose-6-phosphate. The amount of NADP reduced was calculated from the change in optical density with 6.2×10^3 as the molar extinction coefficient of reduced NADP ($NADPH_2$). The assay depends on endogenous glucose-6-phosphate dehydrogenase activity and so gives a minimal value for the enzyme.

6-Phosphogluconate dehydrase. The activity of 6-phosphogluconate dehydrase was determined according to the method of Fraenkel and Horecker (4) by observing the NADP-independent pyruvate formation from 6-phosphogluconate.

TABLE 1. *Bacterial strains*

K-12 strain	Pertinent genotype[a]	Source or reference[b]
C600	*lac*$^-$ ($i^+z^+y^-$) *CR*$^+$ *gal*$^+$ *TL*$^-$	a
JR-11	*lac*$^-$ ($i^+z^+y^-$) *CR*$^+$ *gal*$^-$ *TL*$^-$ *glut*$^-$ *HI*$^-$	b
JR-11-R	*lac*$^-$ ($i^+z^+y^-$) *CR*$^+$ *gal*$^+$ *TL*$^-$ *HI*$^-$	
JR-11-I	*lac*$^-$ ($i^+z^+y^-$) *CR*$^+$ *gal*$^+$ *TL*$^-$ *HI*$^-$	
3.000	*lac*$^+$ ($i^+z^+y^+$) *CR*$^+$ *gal*$^+$	a
LA-12	*lac*$^+$ ($i^+z^+y^+$) *CR*$^-$ *gal*$^+$	c
A-263	*lac*$^+$ ($i^+z^+y^+$) *CR*$^+$ *gal*$^-$	a
A-263-1	*lac*$^+$ ($i^+z^+y^+$) *CR*$^-$ *gal*$^-$	d
A-413	*lac*$^+$ ($i^-z^+y^+/i^-z^+y^+$) *CR*$^+$ *gal*$^-$	a
A-460	*lac*$^+$ ($i^+z^+y^+$) *CR*$^+$ *acet*$^-$	a

[a] The *lac* symbols are identical to those of Loomis and Magasanik (14); CR^-, catabolite insensitivity of the *lac* operon; TL^-, threonine and leucine requirements; $glut^-$, glutamate requirement; $acet^-$, acetate requirement; HI^-, hexose isomerase-negative; gal^-, galactokinase-negative (except strain JR-11 which is galactokinase-positive but unable to ferment galactose).

[b] (a) S. E. Luria; (b) J. Rothman; (c) Loomis and Magasanik (15); Loomis and Magasanik (*in press*). All other strains were isolated during this study.

Tryptophanase. The activity of tryptophanase was determined according to Pardee and Prestidge (22) by observing the formation of indole from L-tryptophan. One unit is defined as that amount which will form 1 mμmole of indole in 1 min at 37 C at *p*H 7.0.

Amylomaltase. Amylomaltase activity was estimated according to Lund and Magasanik (*unpublished data*). Cell extracts were prepared by disruption of a washed, concentrated culture of the various strains in phosphate buffer (*p*H 7.0) with an ultrasonic oscillator (Measuring & Scientific Equipment, Ltd.). The extracts were freed from bacterial debris by centrifugation. To 1 ml of extract in 0.1 M potassium phosphate buffer (*p*H 7.0) was added 0.1 ml of 10% maltose. After exactly 30 min at 37 C, 0.5 ml of 0.114 N KOH and 0.5 ml of a 2% solution of $ZnSO_4 \cdot 7H_2O$ were added. The precipitate was removed by centrifugation, and the supernatant fluid was assayed for glucose by use of the Glucostat reagents (Worthington Biochemical Corp., Freehold, N.J.).

Protein. The concentration of protein was estimated by the method of Lowry et al. (16). Protein synthesis was measured by determining the rate of C^{14}-leucine uptake according to Nakada and Magasanik (20).

RESULTS

Studies on hexose phosphate isomerase-negative mutants. Strain JR-11 was isolated from an

ethylmethane sulfonate (EMS)-mutagenized culture of *Escherichia coli* strain C600 as a mutant which ferments galactose poorly on EMB-galactose plates. It was shown that this phenotype is not related to a mutation in the *gal* operon since, after the introduction of a functional *gal* operon by transduction or F-duction into strain JR-11, the cells are still unable to ferment galactose well (J. Rothman, *personal communication*). The activities of UDP glucose pyrophosphorylase and phosphoglucomutase were found to be normal in strain JR-11. However, the strain was found to contain less than 1% of the wild-type hexose phosphate isomerase activity (5).

It has been shown that T-even phage lysates of bacterial strains lacking UDP glucose are host-restricted (5). Such lysates are termed T*. Normal T4 phage lysates can be made from strain JR-11 if glucose is present in the medium. However, in an amino acid medium lacking glucose, T* phage lysates are produced. It appears that the only biochemical pathway from amino acids to UDP glucose has been lost in this strain.

Although the strain grew well in an amino acid medium, it grew poorly and only after a lag of many days in minimal medium supplemented with the amino acids, threonine and leucine, which the parent strain is known to require. We found that the addition of glutamate to the medium shortened the lag to about 1 day and then allowed reasonable growth of the strain on many carbon sources (Table 2). L-Glutamate (20 μg/ml) was added to the media in all subsequent experiments. However, strain JR-11 was found to grow more slowly than the parent strain on several carbon sources even with added glutamate and was found to grow extremely slowly on fructose, as shown in Table 2. To elucidate the nature of this growth response, we selected derivatives of strain JR-11 for ability to ferment galactose on EMB-galactose agar from an EMS-mutagenized culture (13). We also isolated similar derivatives after infection of strain JR-11 with phage P1 from a lysate of the *gal*$^+$ strain, LA-12. Single colony isolates of the revertant, JR-11-R, and the transductant, JR-11-I, were *gal*$^+$ on the EMB plates. Both strains grow well on glycerol and fructose and require threonine and leucine but not glutamate for growth (Table 2).

The activity of hexose phosphate isomerase in these strains was determined (Table 3). To our surprise, neither strain JR-11-R nor strain JR-11-I had more than 0.1% of the activity of the enzyme found in strain C600. To test for in vivo isomerase activity, T6 phage lysates of strain JR-11-R were prepared. As can be seen in Table 4, T* phage characteristics were found in the lysates of cells grown on amino acids and on amino acids plus fructose. Normal phage lysates were formed when glucose was present in the medium. It appears that strain JR-11-R lacks hexose phosphate isomerase activity in vivo as well as in vitro. The

TABLE 2. *Growth rates of various strains of Escherichia coli*[a]

Carbon source	Strain			
	C600	JR-11	JR-11-R	JR-11-I
Glycerol	90[b]	105	95	95
Glucose	70	115	110	105
Gluconate	78	80	75	75
Fructose	120	>300	120	120
Xylose	75	85	—	—
Succinate	85	85	—	—

[a] The growth rates were determined at 37 C in minimal medium containing 20 μg/ml of L-threonine, 20 μg/ml of L-leucine, 20 μg/ml of L-glutamate, and the various carbon sources at 0.4%. The optical density at 530 mμ was measured on a Klett-Summerson photoelectric colorimeter for at least two generations in the exponential phase of growth.

[b] Mass doubling time (minutes).

TABLE 3. *Hexose phosphate isomerase activity*[a]

Strain	Activity (mμmoles per min per mg of protein)
C600	154
JR-11	0.24
JR-11-R	0.0
JR-11-I	0.09

[a] The activities were determined in extracts of overnight cultures of the strains grown on glycerol minimal medium.

TABLE 4. *Production of T* in strain JR-11-R*[a]

Medium	Efficiency of plating on	
	Shigella dysenteriae	*Escherichia coli* B
Tryptone	1.0	10^{-4}
Tryptone + 0.4% glucose	1.0	0.8
Tryptone + 0.4% fructose	1.0	10^{-4}

[a] Phage T6 lysates of strain JR-11-R in the various media were prepared by incubating 2×10^7 T6 phage with 3×10^8 bacteria at 37 C for 10 min, collecting the bacteria by centrifugation, and resuspending them at 3×10^4 bacteria per milliliter in the various media. After 80 min at 37 C, a drop of chloroform was added. Dilutions were made for titering phage on *S. dysenteriae* 16 and *E. coli* B.

biochemical basis for poor galactose fermentation and lack of growth on fructose in strain JR-11 is not understood, but does not appear to be related to hexose phosphate isomerase activity. In any case, strains JR-11-R and JR-11-I appear completely normal except for the loss of hexose phosphate isomerase activity and the concomitant inability to form glucose derivatives from carbon sources not containing glucose or galactose.

A similar hexose phosphate isomerase-negative mutant of *Salmonella typhimurium* has been isolated (4). In contrast to the isomerase-negative strains of *E. coli*, the *Salmonella* mutant grows very slowly on glucose. In an effort to determine the basis for this difference, we investigated the Entner-Doudoroff pathway. *E. coli* is known to contain 2-keto-3-deoxy 6-phosphogluconate aldolase, the second enzyme of the pathway (12). We found that *E. coli* forms 6-phosphogluconate dehydrase, the first enzyme of the pathway, when grown on glucose, gluconate, or glycerol (Table 5). Thus, it appears that *E. coli* forms the Entner-Doudoroff enzymes constitutively in contrast to *Salmonella*, which forms 6-phosphogluconate dehydrase only when grown on gluconate. The utilization of this pathway for glucose metabolism in the isomerase-negative mutants of *E. coli* may account for the relatively rapid growth on glucose.

Catabolite repression in strains JR-11, JR-11-R, and JR-11-I. We determined the differential rate of β-galactosidase synthesis in strains C600, JR-11, JR-11-R, and JR-11-I after induction in minimal medium minus a source of nitrogen, in the presence of various carbon sources. The cultures were all preadapted to growth on the test carbon source. Thus, complications due to transient repression of β-galactosidase were not present in these experiments (Loomis and Magasanik, *in press*). Likewise, IPTG was present in all experiments at 10^{-3} M concentration, a concentration known to give optimal induction of cells growing in glucose-containing medium (14). The repression observed under these conditions is completely relieved by a mutation in a gene, *CR*, which has been shown to determine catabolite repression specific to the *lac* operon (15; Loomis and Magasanik, *in press*).

Under these conditions, extremely strong catabolite repression of β-galactosidase is exerted by gluconate, xylose, lactate, succinate, and fructose in all strains tested (Table 6). These carbon sources cannot give rise to either glucose or galactose nor to their derivatives in the isomerase-negative strains. Thus, it appears that catabolite repression of β-galactosidase can result from a high concentration of compounds other than those related to glucose or galactose.

If one assumes that lactate gives rise to a catabolite which represses β-galactosidase independently of hexose phosphate isomerase activity, one would expect glycerol to give rise to the same catabolite independently of hexose phosphate isomerase activity. However, it can be seen in Table 6 that, although glycerol in the absence of a nitrogen source elicits strong repression of β-galactosidase in the wild-type strain, it does not repress β-galactosidase in the isomerase-negative mutants. The weight of evidence, however, suggests that the lack of repression by glycerol in hexose phosphate isomerase-negative mutants is not due to the fact that the formation of glucose-6-phosphate is blocked in these mutants. At present, the problem remains a mystery.

Nevertheless, we decided to determine whether glycerol would give rise to catabolite repression of tryptophanase and amylomaltase in strain JR-11 in the absence of a nitrogen source. It can be seen (Table 7) that, although gluconate represses amylomaltase strongly in both strain

TABLE 5. *6-Phosphogluconate dehydrase activity*[a]

Strain	Carbon source	Activity (mμmoles per min per mg of protein)
3.000	Glucose	78
3.000	Gluconate	180
C600	Glycerol	50
LA-12	Glucose	62
LA-12	Gluconate	156

[a] The strains were grown overnight in minimal medium with the various carbon sources. The bacteria were collected by centrifugation, washed, and suspended in glycylglycine-HCl buffer (*p*H 7.4). The enzyme was measured in sonic extracts as described in Materials and Methods.

TABLE 6. *Catabolite repression of β-galactosidase*[a]

Strain	Gluconate	Fructose	Xylose	Glycerol	Lactate	Succinate
C600	4	1	1	7	4	14
Jr-11	7	—	2	90	4	13
JR-11-R	1	5	—	112	1	—
JR-11-I	—	—	—	80	—	—

[a] Values are the differential rate of β-galactosidase synthesis in minimal medium lacking a nitrogen source, containing various carbon sources at 0.4% concentration. The rate in the absence of both a carbon and a nitrogen source is taken as 100. The rate of synthesis was determined over a period of 80 min after the addition of 10^{-3} M IPTG.

TABLE 7. *Catabolite repression of tryptophanase and amylomaltase*

Strain	Carbon source	Tryptophanase[a]		Amylomaltase[b]
		$+NH_4^+$	$-NH_4^+$	
C600	Glycerol	100	—	7
	Glucose	26	—	—
	Gluconate	37	—	0
JR-11	Glycerol	100	20	81
	Glucose	32	—	—
	Gluconate	43	—	0

[a] The values in nitrogen-containing medium are the relative rates of enzyme synthesis after induction with 1 mg/ml of L-tryptophan. The rate with glycerol as carbon source is taken as 100. The rate was determined over a period of 60 min. All values are the average of at least two determinations. The values in the absence of a nitrogen source are the relative differential rates of synthesis. The rate when no carbon source is present is taken as 100. The rate was determined over a period of 80 min.

[b] Values are the relative specific activities of amylomaltase after incubation overnight in the presence of 0.4% maltose. The specific activity of a culture incubated overnight without added carbon source is taken as 100

JR-11 and the parent strain C600, glycerol only represses this enzyme in the parent strain. Glycerol exerts strong repression of tryptophanase in strain JR-11 under this condition. It appears that the catabolite related to tryptophanase can be formed in the mutant from both glycerol and gluconate, and the catabolite related to amylomaltase and β-galactosidase can be formed in the mutant from gluconate but not from glycerol.

Repression by glucose in strain JR-11. Since we found that strain JR-11 grows more slowly on glucose than on glycerol (Table 2), we wondered whether glucose would give rise to catabolite repression of β-galactosidase in a growing culture. The fully induced differential rate of β-galactosidase synthesis in a culture of strain JR-11 was found to be reduced by the presence of glucose in the medium to 55% of the differential rate in glycerol-containing medium. It appears that glucose can give rise to catabolite repression of β-galactosidase, even when the growth rate on glucose is reduced by loss of hexose phosphate isomerase activity.

When the oxidation of glucose to gluconate in *Aerobacter aerogenes* is impaired by mutation, glucose no longer represses β-galactosidase (21). Since an impairment in utilization of the pentose shunt, but not a block in the Embden-Meyerhof pathway, reduces catabolite repression of β-galactosidase, it seems that the specific catabolite may be a derivative of the pentose shunt.

Studies in a mutant unable to form acetyl-coenzyme A (CoA). To define further the compounds involved in catabolite repression of β-galactosidase, we investigated the repression elicited by glucose in a mutant unable to form compounds of the citric acid cycle in the absence of exogenous acetate.

E. coli strain A-460 requires acetate for growth and has been shown to have only 0.5% of the wild-type lipoic reductase-transacetylase activity (6). A culture of this strain growing exponentially in glycerol minimal medium with 0.4% acetate was induced with 10^{-3} M IPTG, and glucose was added to one half of the culture. The rate of β-galactosidase synthesis was reduced in the glucose medium to 50% of the rate in the glycerol medium. To exclude the Krebs cycle intermediates from a role in catabolite repression of β-galactosidase, a glucose-grown culture of strain A-460 was collected on membranes, washed, and resuspended in minimal medium, but acetate was omitted. Glucose was added to one half of the culture, and the other half remained free from a carbon source. The cells could not grow under these conditions. It was found that the differential rate of β-galactosidase synthesis was reduced to 5% of the rate in the carbon-free culture by the presence of glucose.

It has been shown that, when the concentration of acetyl-CoA is low, very little of the 4-carbon compounds of the Krebs cycle can be formed (1). Thus, it appears that neither compounds of the Krebs cycle nor the derivatives of these compounds are required for complete catabolite repression of β-galactosidase.

Apparent repression by galactose. We have shown that several carbon sources cause catabolite repression of β-galactosidase in a mutant in which none of the galactose compounds can be formed from these carbon sources. Therefore, we investigated the repression elicited by galactose in strains able and unable to metabolize galactose.

Unlike McFall and Mandelstam (17), we did not observe repression by galactose of β-galactosidase in growing cultures of a galactose-positive strain, 3.000. McFall and Mandelstam utilized a strain, 58-161, which had been selected for strong repression of β-galactosidase by glucose, and it is conceivable that galactose is also a better source of catabolites in strain 58-161 than in strain 3.000. We did observe a severe repression by galactose of β-galactosidase when anabolism was reduced by the lack of a nitrogen source in the wild-type strain, 3.000, but not in the strain carrying a muta-

tion in the *CR* gene, LA-12G (Table 8). A mutation in the *CR* gene has been shown to render a strain insensitive to catabolite repression of the *lac* operon by several carbon sources even under conditions of nitrogen starvation (15). Therefore, we conclude that the repression elicited by galactose in the wild-type strain is dependent on a functional *CR* gene and appears to be an expression of catabolite repression.

In agreement with the observations of McFall and Mandelstam (17), galactose was found to give rise to repression of β-galactosidase in a galactokinase-negative mutant, A-263 (Table 8; Fig. 1). However, repression of β-galactosidase by galactose was also observed in a CR^- derivative of the galactokinase-negative mutant, A-263-1 (Table 8; Fig. 1). Thus, the repression of β-galactosidase elicited in these mutants, unable to metabolize galactose, appears to be independent of the gene which determines catabolite sensitivity specific to the *lac* operon.

Galactose appears to repress β-galactosidase in growing cultures of a galactose-negative strain but not in growing cultures of a wild-type strain. It has been shown that the internal concentration of galactose is much higher in galactokinase-negative cells than in wild-type cells (7). The repression of β-galactosidase in galactokinase negative strains growing in the presence of galactose may result from this increased internal galactose, per se.

TABLE 8. *Repression by galactose*

Strain	Pertinent genotype		Rate of β-galactosidase synthesis in galactose-containing medium	
	lac	*gal*	$+NH_4^+$ [a]	$-NH_4^+$ [b]
3.000	$i^+z^+y^+CR^+$	+	100	1
LA-12G	$i^+z^+y^+CR^-$	+	100	106
A-263	$i^+z^+y^+CR^+$	−	58	—
A-263-1	$i^+z^+y^+CR^-$	−	56	—
A-413	$F'i^-z^+y^+/i^-z^+y^+$ CR^+	−	100	—

[a] The values are the differential rate of enzyme synthesis in minimal medium containing 0.1% Casamino Acids, 0.4% glycerol, 2×10^{-2} M galactose, and 5×10^{-4} M IPTG. The differential rate of synthesis in the absence of galactose is taken as 100, and was similar in all strains. The rate was determined over a period of 90 min. The galactose-negative strains lack galactokinase activity.

[b] The values are the differential rate of enzyme synthesis in minimal medium minus NH_4^+, containing 2×10^{-2} M galactose and 10^{-3} M IPTG. The differential rate in the absence of a carbon source is taken as 100 and was similar in both strains. The rate was determined over a 90-min period.

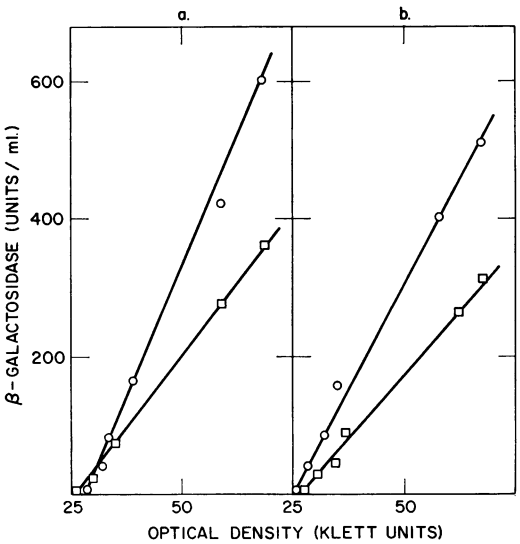

FIG. 1. *Repression by galactose in CR^+ and CR^- galactose-negative strains. A culture of cells in the exponential phase of growth in glycerol minimal medium was induced with 5×10^{-4} M IPTG. The culture was immediately divided and 10^{-2} M galactose was added to one half. Samples were taken for determination of β-galactosidase activity. The optical density at 530 mμ was observed on a Klett-Summerson photoelectric colorimeter. (a) Strain A-263 lac^+ ($i^+z^+y^+$ CR^+) galactokinase$^-$; (b) strain A-263-1 lac^+ ($i^+z^+y^+$ CR^-) galactokinase$^-$.*

When glucose is added to fully induced lac^+ cells growing on carbon sources other than glucose, β-galactosidase synthesis is strongly repressed for about 40 min. After this period of growth in the presence of glucose, β-galactosidase is synthesized at the rate determined by catabolite repression in the particular strain. The repression by galactose in galactokinase-negative mutants does not appear to be a case of this transient repression, since the repression was relatively weak and appeared constant during 80 min of growth in the presence of galactose (Fig. 1).

Glucose is also able to repress severely β-galactosidase synthesis in glucose-grown cells when induced by low levels of inducers. This severe repression can be overcome in β-galactoside permease-positive cells by a short period of preinduction of the *lac* operon (Loomis and Magasanik, *in press*). It appears that the presence of glucose in the medium reduces the internal concentration of inducer so that only partial induction of the *lac* operon can occur. The ability

to concentrate inducer in the cells, i.e., permease activity, overcomes this effect of glucose (9; Loomis and Magasanik, *in press*). The repression by galactose of β-galactosidase in galactokinase-negative mutants is not relieved even after 1 hr of induction of the *lac* operon. Since β-galactosidase activity and, presumably, permease activity increased markedly during this period, it appears that increased permease activity does not relieve the repression by galactose in these galactose-negative mutants. It has been shown that galactose inhibits the entry of galactosides into induced permease-positive cells (11). Thus, galactose may give rise to repression of β-galactosidase in induced cells of a galactokinase-negative strain by competing with inducer for entry into the cells. Therefore, β-galactosidase synthesis in a galactokinase-negative strain constitutive for β-galactosidase might not be repressed by galactose, since the rate of β-galactosidase synthesis is independent of internal concentration of inducer in such a strain. When the differential rate of β-galactosidase synthesis was determined in strain A-413 (F $i^-z^+y^+/i^-z^+y^+$, gal^-), it was found that the presence of galactose in the medium did not repress the enzyme in this *lac* constitutive strain (Table 8). Thus, it appears that galactose gives rise to repression of β-galactosidase in galactokinase-negative mutants by an inhibition of induction and not by catabolite repression of the operon.

DISCUSSION

When anabolism of wild-type *E. coli* is reduced by nitrogen starvation, the presence of any carbon source in the medium reduces the differential rate of β-galactosidase synthesis by a factor of more than 10 (19). It is thought that the concentration of one or more catabolites in the cells determines the degree of repression of a specific operon (18).

Our results with the hexose phosphate isomerase-negative mutant indicate that catabolite repression of the *lac* operon does not require glucose, galactose, gluconate, or their direct derivatives, since these compounds cannot be formed by any known pathway from fructose, lactate, xylose, or succinate in an isomerase-negative strain, and yet a strong repression of β-galactosidase by these compounds can be observed when anabolism is reduced. Likewise, the compounds related to the Krebs cycle do not appear to be required for catabolite repression of the *lac* operon, since glucose gives rise to strong repression in a mutant under conditions where the formation of Krebs cycle compounds is severely restricted.

McFall and Mandelstam (17) reported repression of β-galactosidase by galactose in mutant strains unable to grow on galactose. They suggested that the degree of catabolite repression of the *lac* operon is determined by the concentration of galactose compounds. We have presented evidence that the repression by galactose observed in galactokinase-negative strains is the result of an inhibition of induction which is independent of the gene, *CR*, which determines catabolite repression specific to the *lac* operon. Thus, galactose does not appear to be involved in catabolite repression of β-galactosidase.

If catabolite repression of the *lac* operon is effected by a single compound, we suggest that the compound is related to the pentoses and trioses of intermediary metabolism.

ACKNOWLEDGMENTS

One of us (W.F.L.) has been a trainee under Public Health Service training grant 5T1 GM 602 from the National Institutes of Health to the Department of Biology, Massachusetts Institute of Technology. This investigation was supported by National Science Foundation grant GB-265 and Public Health Service grant GM-07446.

LITERATURE CITED

1. CANOVAS, J., AND H. KORNBERG. 1965. Fine control of phosphopyruvate carboxylase activity in *Escherichia coli*. Biochim. Biophys. Acta **96:** 169–172.
2. COHN, M., AND K. HORIBATA. 1959. Inhibition by glucose of the induced synthesis of the β-galactoside-enzyme system of *Escherichia coli*. Analysis of maintenance. J. Bacteriol. **78:**601–612.
3. COHN, M., AND K. HORIBATA. 1959. Physiology of the inhibition by glucose of the induced synthesis of the β-galactoside-enzyme system of *Escherichia coli*. J. Bacteriol. **78:**624–635.
4. FRAENKEL, D., AND B. HORECKER. 1964. Pathways of D-glucose metabolism in *Salmonella typhimurium*. J. Biol. Chem. **239:**2765–2771.
5. HATTMAN, S. 1964. The functioning of T-even phages with unglucosylated DNA in restricting *Escherichia coli* host cells. Virology **24:**333–348.
6. HENNING, V., AND C. HERZ. 1964. Ein Strukturgen-Komplex für den Pyruvat-Dehydrogenase-Komplex von *Escherichia coli* K12. Z. Vererbungslehre **95:**260–275.
7. HORECKER, B., J. THOMAS, AND J. MONOD. 1960. Galactose transport in *Escherichia coli*. I. General properties as studied in galactokinaseless mutant. J. Biol. Chem. **235:**1580–1585.
8. KENNEL, D., AND B. MAGASANIK. 1964. The control of the rate of enzyme synthesis in *Aerobacter aerogenes*. Biochim. Biophys. Acta **81:** 418–434.
9. KEPES, A. 1960. Etudes cinetiques sur la galactoside-permease d'*Escherichia coli*. Biochim. Biophys. Acta **40:**70–84.

10. KESSLER, D., AND H. RICKENBERG. 1964. A new method for the selection of mutants of *Escherichia coli* forming β-galactosidase constitutively. Biochim. Biophys. Acta **90**:609–610.
11. KOCH, A. 1964. The role of permease in transport. Biochim. Biophys. Acta **77**:177–200.
12. KOVACHEVICH, R., AND W. WOOD. 1955. Carbohydrate metabolism by *Pseudomanas fluorescens*. IV. Purification and properties of 2-keto-3-deoxy-6-phosphogluconate aldolase. J. Biol. Chem. **213**:751–767.
13. LIN, E. C. C., S. A. LERNER, AND S. E. JORGENSEN. 1962. A method for isolating constitutive mutants for carbohydrate-catabolizing enzymes. Biochim. Biophys. Acta **60**:422–424.
14. LOOMIS, W. F., JR., AND B. MAGASANIK. 1964. The relation of catabolite repression to the induction system for β-galactosidase in *Escherichia coli*. J. Mol. Biol. **8**:417–426.
15. LOOMIS, W. F., JR., AND B. MAGASANIK. 1965. Genetic control of catabolite repression of the *lac* operon in *Escherichia coli*. Biochem. Biophys. Res. Commun. **20**:230–234.
16. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
17. MCFALL, E., AND J. MANDELSTAM. 1963. Specific metabolic repression of three induced enzymes in *Escherichia coli*. Biochem. J. **89**:391–398.
18. MAGASANIK, B. 1961. Catabolite repression. Cold Spring Harbor Symp. Quant. Biol. **26**:249–256.
19. MANDELSTAM, J. 1962. The repression of constitutive β-galactosidase in *Escherichia coli* by glucose and other carbon sources. Biochem. J. **82**:489–493.
20. NAKADA, D., AND B. MAGASANIK. 1964. The roles of inducer and catabolite repressor in the synthesis of β-galactosidase by *Escherichia coli*. J. Mol. Biol. **8**:105–127.
21. NEIDHARDT, F. 1960. Mutant of *Aerobacter aerogenes* lacking glucose repression. J. Bacteriol. **80**:536–543.
22. PARDEE, A., AND L. PRESTIDGE. 1961. The initial kinetics of enzyme induction. Biochim. Biophys. Acta **49**:77–88.