Role of the Galactose Pathway in the Regulation of β -Galactosidase

KENNETH PAIGEN

Department of Experimental Biology, Roswell Park Memorial Institute, New York State Department of Health,
Buffalo, New York

Received for publication 31 May 1966

ABSTRACT

PAIGEN, KENNETH (Roswell Park Memorial Institute, Buffalo, N.Y.). Role of the galactose pathway in the regulation of β -galactosidase. *J. Bacteriol.* 92:1394-1403. 1966. —Galactose and its metabolites, galactose-1-phosphate, uridine diphosphogalactose, and uridine diphosphoglucose, as well as metabolites derived from uridine diphosphoglucose, were tested for their role in the regulation of β -galactosidase. In cultures of wild-type *Escherichia coli* strains K-12 and B, exogenous galactose was no more effective as a repressor than were other carbon sources. Exogenous galactose also did not repress β -galactosidase when added to mutants which can accumulate intracellular galactose or galactose-1-phosphate, indicating that these compounds do not repress. In such strains, repression of β -galactosidase formation did occur if galactose was added in the presence of another metabolizable carbon source. This repression is presumably a consequence of the growth inhibition which follows the accumulation of these compounds, and the general catabolite repression which develops during growth inhibition. Exogenous galactose did repress β -galactosidase in a mutant which accumulates uridine diphosphogalactose. This appears to result from a combination of several factors. These include a general inhibition of protein synthesis through depletion of the uridine triphosphate pool, catabolite inhibition as a consequence of growth inhibition, as well as a specific inhibition of β -galactosidase formation. Glucose repression of β -galactosidase was normal in a mutant strain blocked in the formation of uridine diphosphoglucose from uridine triphosphate and glucose-1-phosphate, indicating that neither uridine diphosphoglucose nor any compound uniquely derived from it functions as the hypothetical catabolite repressor. It is concluded that at least two separate mechanisms exist for the endogenous repression of β -galactosidase in *E. coli*. One is exerted by uridine diphosphogalactose or its metabolic product; the other, by the generalized catabolite repressor which is still formed in strains unable to make uridine diphosphogalactose or uridine diphosphoglucose.

The induction and repression of the β -galactosidase of *Escherichia coli* has been a favored experimental system in the study of genetic regulation for some years. During this period, the structural requirements for inducer activity by small molecules have been defined in considerable detail (2, 5, 9, 10, 18, 19, 23-25). In contrast to this, very little is known about the structural requirements for repressor activity, and the identity of the endogenous repressor(s) of this enzyme has not been determined. We know only that the formation of the enzyme is inhibited both by the catabolic products of intermediary metabolism (5, 16), and by a series of compounds, phenylthiogalactoside (9, 18), *o*-nitrophenyl fucoside (19), and D(+)-fucose (28), which do not normally occur in *E. coli*. In the case of catabolite repression, we also do not know whether the effective

compound is specific in regulating only the lactose operon, or is general and controls the concentration of many carbohydrate-utilizing enzymes.

The two products of the action of β -galactosidase on its natural substrate, lactose, are glucose and galactose. [Although this enzyme does catalyze the formation of other galactosides by transgalactosylation reactions (27), these products must presumably be broken down to free galactose before they can be used to provide cellular energy. No route for the utilization of galactosides is known which does not pass through free galactose and the galactose-metabolizing enzymes.] It is the purpose of this paper to consider whether one of these products, galactose, or any of its further metabolites, is the elusive natural repressor and provides an end-product repression analogous to that seen in anabolic pathways. For this

purpose, the induction of β -galactosidase was examined in wild-type strains of *E. coli* and in mutants defective in the various enzymes of the galactose pathway. From the behavior of these strains, it appears that uridine diphosphogalactose (UDPgal), but not galactose or galactose-1-phosphate (gal-1-P), functions as a repressor, and that this control is distinct from the catabolite repression produced by the general metabolic pool.

MATERIALS AND METHODS

Bacterial strains. The parent strain of *E. coli* K-12 used was an F⁻ prototroph (W3110); the various galactose-negative mutants were derived from it. The transferaseless strain $K^+ T^- E^+$ (gal 4: 3104) and the kinaseless-transferaseless double mutant $K^- T^- E^+$ (gal 1,2: W3350) were obtained by transduction of the respective mutant genes into the W3110 parent by λ transducing phage. The mutants were originally isolated by Esther Lederberg, and were obtained from J. J. Weigle. The epimeraseless strain $K^+ T^+ E^-$ (gal 22: W3805) and the K-12 mutant lacking the enzyme UDPglu synthetase (W4597) were obtained from Dr. Lederberg. Their enzymatic constitution was originally determined by Kurahashi and associates (7, 14, 15). The *met*⁺ revertant of *E. coli* strain 58-161 ($T^- L^- B^- mer^-$) was obtained from J. Mandelstam, and *E. coli* B, from A. D. Hershey.

Growth experiments. For all experiments cells were grown at 37°C to about 2×10^8 cells per milliliter, centrifuged, washed with carbon-free synthetic medium, and resuspended to the same cell density. Cell growth during the experimental period is expressed as A_t/A_0 , where A_t and A_0 represent the absorbancy at 550 m μ of the culture at times t and 0 . Optical measurements were made in a Zeiss PMQII spectrophotometer in a 1-cm cell. Enzyme yield is expressed as $(E_t - E_0)/A_0$, where E_t and E_0 represent the number of units of enzyme per milliliter present at times t and 0 . The synthetic medium used, M95, is a simple salts mixture with the composition of Anderson's (1) M9 medium, except for a reduction of the phosphate and NH_4Cl concentrations to one-fifth of their original values. Unless noted otherwise, the carbon source was 0.2% glycerol. When other carbon sources were used, they were added at 0.2 or 0.25% concentration. For the growth of strain 58-161, the medium was further supplemented with 20 μ g/ml each of the L isomers of threonine, leucine, isoleucine, and valine, and with 0.5 μ g/ml of thiamine-HCl. In all other respects, the experimental procedures employed were as described previously (21). Isopropyl thiogalactoside (IPTG) at 5×10^{-4} M was used as inducer for β -galactosidase, and D(+)-fucose at 5×10^{-3} M was used as inducer for the galactose operon. Uracil was added at a concentration of 10^{-4} M in some experiments.

Enzyme measurements. Galactokinase was measured by use of the C^{14} -galactose-Pb precipitation procedure. β -Galactosidase was measured by the hydrolysis of *o*-nitrophenyl galactoside. A unit of β -galactosidase is defined here as that quantity of enzyme which forms 1 μ mole of *o*-nitrophenol per minute at 30°C.

The details of these procedures as conducted in this laboratory have been described previously (21).

Amino acid uptake. Cultures were exposed to 10^{-4} M C^{14} -leucine at a specific activity of 1.1 μ c/ μ mole. At various times, duplicate 1.0-ml samples were taken and were heated in a boiling-water bath for 2 min. The samples were then chilled, and 1.0 ml of cold 0.3 N trichloroacetic acid was added. The mixtures were filtered onto prewetted membrane discs (HA; Millipore Filter Corp., Bedford, Mass.), and were washed four times with 5-ml portions of cold trichloroacetic acid, twice with water, and twice with ether. The discs were then air-dried and counted in a scintillation counter. Uptake is expressed as millimicromoles of leucine incorporated, after correction for the blank, divided by the initial absorbance (A_0) of the culture.

Materials. Radioactive L-leucine was obtained from Volk Radiochemical Co., Skokie, Ill., D(+)-fucose from K and K Laboratories, Jamaica, N.Y., glucose-free galactose from Sigma Chemical Co., St. Louis, Mo., isopropyl- β -thiogalactoside (IPTG) from Calbiochem, and C^{14} -galactose from the U.S. National Bureau of Standards.

RESULTS

Induction in growing cultures of wild-type strains. The addition of any metabolizable carbon compound to a culture of *E. coli* always results in some decrease in the differential rate of β -galactosidase synthesis. This occurs presumably because the hypothetical endogenous repressor can be formed during the normal course of metabolism from any carbon source capable of feeding the general metabolic pool. The first question to be asked, therefore, is whether galactose is unusually effective in this regard, or whether its action is comparable to that of other carbon sources which can be utilized at similar rates.

Figure 1a shows the induction of β -galactosidase in a wild-type strain of *E. coli* K-12 (W3110) growing upon various carbon sources in the presence of IPTG as a nonmetabolizable inducer of the enzyme. Among the four substrates tested in this experiment, the differential rate of β -galactosidase synthesis was highest in lactate and lowest in glucose; the rates in galactose- and glycerol-grown cultures were intermediate. When tested in combination with other carbon substrates, galactose again did not show an unusual capacity to repress. Its presence, when added to lactate, had no more effect than did that of glycerol (Fig. 1b). When galactose, glycerol, and glucose combinations were tested, galactose again failed to exert a specific effect (Fig. 1c). Moreover, when acetate, succinate, or gluconate was used as a single carbon source, the rate of induction of β -galactosidase was the same as that observed with galactose (22).

Similar results were obtained with *E. coli* B

FIG. 1. β -Galactosidase induction in gal^+ cells grown in various carbon sources. Cells pregrown in glycerol were harvested and resuspended in each of the indicated carbon sources of 0.25% concentration with IPTG present. The yield of β -galactosidase was calculated as described in Materials and Methods.

(Table 1), indicating that in this strain also galactose was no more effective as a repressor of β -galactosidase than were other carbon sources capable of supporting similar rates of growth.

Taken together, the results obtained with wild-type cells of these two strains of *E. coli* indicate that exogenous glucose is a more effective repressing agent than is exogenous galactose, and that galactose is not remarkably different from other carbon sources.

Roles of galactose and *gal-1-P*. The pathway of galactose metabolism of *E. coli*, together with some related reactions, is indicated in Fig. 2. The enzymes for the three steps, galactokinase (K), galactose-1-phosphate uridyl transferase (T), and uridine diphosphogalactose-4-epimerase (E), are determined by a single coordinately regulated operon (4). No other pathway for galactose metabolism exists in *E. coli* K-12 (3, 12). Moreover, *E. coli* can form an active galactose permease (12). Hence, in the presence of exogenous galactose, mutants defective in the various steps of the galactose pathway accumulate the intermediates whose further metabolism is blocked (6, 14, 26, 29). This effect results in the accumulation of free intracellular galactose in $gal K^- T^-$ cells, $gal-1-P$ in the case of $gal T^-$ cells which are missing the transferase, and UDPgal in the case of $gal E^-$ cells lacking epimerase.

The induction of β -galactosidase was therefore examined in such mutants in the presence and

TABLE 1. β -Galactosidase induction in *Escherichia coli* B^a

Carbon source	Expt I		Expt II	
	Growth rate	Inducibility	Growth rate	Inducibility
Glucose.....	0.76	640	0.80	720
Glycerol.....	0.63	1650	0.69	1940
Galactose.....	0.54	900	0.48	850
Glycerol + galactose..	0.62	920	0.68	1110
Glycerol + glucose...	0.76	520	0.77	590
Galactose + glucose..	0.82	430	0.80	530

^a Cells were pregrown in glycerol medium to 1.5×10^8 cells per milliliter, harvested, and resuspended at an initial density of 10^8 cells per milliliter in 0.25% of each carbon source in M95 medium containing IPTG. Growth and enzyme induction were followed subsequently for 2.5 and 3.5 hr, respectively, for experiments I and II. Growth rate is expressed as the rate constant taken from the growth curves during this period. Inducibility is expressed as the number of units of β -galactosidase formed per unit increase in absorbance (see Materials and Methods). All induction curves were linear.

absence of exogenous galactose to determine whether any of these intermediates are capable of repressing this enzyme. In doing so, it is assumed that accumulation of the intermediates occurs under the experimental conditions used. The re-

sults obtained were consistent with this assumption.

The effects of galactose and gal-1-P accumulation were tested in *gal K⁻T⁻* and *gal K⁺T⁻* cells. In the absence of any other carbon source, galactose addition had no effect (Fig. 3), indicating that neither galactose nor gal-1-P is the natural repressor.

As a further test for a possible role of gal-1-P in β -galactosidase regulation, the induction of this enzyme has been compared in *gal* T^- cells with high and low galactokinase activities. *Gal* T^- cells were pregrown in glycerol plus D(+)-fucose (a nonmetabolizable inducer of the galactose operon), which results in a high level of activity of galactokinase (3), and in glucose, which represses

galactokinase formation. The induction of β -galactosidase was then measured in the presence and absence of galactose after transfer of these cells to fresh medium containing IPTG but lacking a metabolizable carbon source. As shown in Fig. 4, an increase in the activity of galactokinase did not predispose the cells to repression of β -galactosidase by galactose. The analogous experiment with *gal K⁻T⁻* cells was not performed. Galactose permease is constitutive in *gal K⁻* cells, and its formation is not influenced by the presence of an inducer (11). Thus, it is not possible in *gal K⁻* cells to vary the internal level of galactose by growing the cells under conditions which increase the concentration of galactose permease.

There is no obvious explanation for the differences in enzyme-forming capacity observed among the various cultures used for the experiments of Fig. 3 and 4. While such differences are

FIG. 2. *Pathway of galactose metabolism in Escherichia coli.*

FIG. 3. Effect of galactose on gal $K^{-}T^{-}$ and gal T^{-} under starvation conditions. Induction was carried out in the absence of a metabolizable carbon source. Symbols: $\circ$, gal $K^{-}T^{-}$; $\bullet$, plus galactose; Δ , gal T^{-} without galactose; $\blacktriangle$, plus galactose.

FIG. 4. Effect of galactose on gal T⁻ after preinduction of the galactose pathway. Cells of gal T⁻ were pregrown with glucose or with a mixture of glycerol plus fucose. The latter had an initial galactokinase activity (0.03 μ mole of gal-1-P formed per hour per OD of cells) 5.7 times that of the former. β -Galactosidase induction was then carried out in the absence of a metabolizable carbon source. Symbols: $\circ$, low galactokinase cells without galactose; $\bullet$, plus galactose, $\triangle$, high galactokinase cells without galactose; $\blacktriangle$, plus galactose.

of ultimate interest, for the present it is sufficient to know that the addition of galactose was without effect under any circumstance.

Similar experiments with the two mutant strains were also carried out in the presence of a metabolizable carbon source (Fig. 5). With glycerol as the carbon source, the addition of galactose reduced the differential rate of β -galactosidase synthesis somewhat in *gal K⁻T⁻* cells and produced a slight, but reproducible, growth inhibition as well. In *gal T⁻* cells, both the repression and the growth inhibition caused by galactose were more intense. Inhibition of growth as a consequence of the accumulation of phosphorylated galactose compounds has been described previously (6, 8, 14, 20, 26, 29). The repression produced in these strains by galactose is correlated with its ability to inhibit growth. It has also been shown that growth inhibition by itself is sufficient to cause catabolite repression by virtue of a non-specific accumulation of intermediary metabolites (21). These results should not, therefore, be construed as evidence for the participation of galactose or galactose-1-phosphate in a specific repression of β -galactosidase.

Role of UDPgal. The previous experiments argue against the identification of either galactose or gal-1-P as a repressor of β -galactosidase. The other low molecular weight galactosyl compound known to occur in *E. coli*, and the third member of the galactose fermentation pathway, is UDPgal. The possibility that it participates in the regulation of β -galactosidase was tested with strain W3805, which lacks the enzyme uridine diphosphogalactose-4-epimerase, and hence is unable to carry out the reversible epimerization of UDPgal and uridine diphosphoglucose (UDPglu). In the presence of exogenous galactose, this strain accumulates UDPgal.

FIG. 5. Galactose repression in *gal K⁻T⁻* and *gal T⁻* during growth in glycerol. Symbols: $\circ$, *gal K⁻T⁻* without galactose; $\bullet$, plus galactose; Δ , *gal T⁻* without galactose; $\blacktriangle$, plus galactose.

The ability of galactose to repress this strain was tested during growth in glycerol, when a low level of the galactose enzymes is present, and during growth in glycerol plus D(+)-fucose, when a high level of the galactose enzymes is formed. The result of a typical experiment is presented in Fig. 6. The ability of galactose to repress β -galactosidase and to inhibit growth was much greater when a high level of the galactose enzymes was present. The growth inhibition of epimeraseless strains by galactose has been reported previously (see references above).

These results do not distinguish whether UDPgal acts both as a growth inhibitor and as a repressor under these circumstances, or whether the repression is a secondary consequence of the growth inhibition.

The ability of exogenous galactose to repress β -galactosidase synthesis in the epimeraseless mutant was therefore tested when high and low levels of the galactose enzymes were present and no other carbon source was available to the cells (Fig. 7). In contrast to the kinaseless and transferaseless mutants, which were not repressed by galactose under starvation conditions, the epimeraseless mutant was strongly repressed. Furthermore, the repression was more intense in cells with a high level of the galactose enzymes. These results suggest that UDPgal might play a direct role in the regulation of β -galactosidase synthesis.

Maintenance of the uridine triphosphate (UTP) pool. Before accepting the conclusion that

FIG. 6. Galactose repression of *gal E⁻* during growth. Cells were pregrown with glycerol as carbon source in the presence or absence of fucose. The presence of fucose caused a 17.4-fold increase in galactokinase activity (to 0.52 μ mole of gal-1-P formed per hour per OD of cells). β -Galactosidase induction was then carried out with the cells suspended in a fresh medium of the same composition as that used during pregrowth but with the further addition of IPTG. Symbols: $\circ$, low galactokinase without galactose; $\bullet$, plus galactose; Δ , high galactokinase without galactose; $\blacktriangle$, plus galactose.

FIG. 7. Galactose repression of gal E^- under starvation conditions. The cultures, experimental protocol, and symbols are the same as for Fig. 6 except for the omission of glycerol and fucose from the medium during the period of β -galactosidase induction.

UDPgal is a specific repressor of β -galactosidase, we must take into account the possibility that the addition of galactose to an epimeraseless mutant not only results in an accumulation of UDPgal, but also depletes the UTP pool under conditions where the de novo synthesis of pyrimidines is impaired. Depletion of the UTP pool could result in apparent repression by preventing the resynthesis of messenger ribonucleic acid (RNA).

As shown in Fig. 8, the effect of galactose addition is reversed by the further addition of exogenous uracil, indicating that inhibition of RNA synthesis is indeed a factor in the mechanism of galactose inhibition. This experiment was performed with cells previously adapted for the conversion of uracil to UTP and possessing elevated levels of galactokinase and galactotransferase. It would also be expected that, if galactose functions to deplete the UTP pool in an epimeraseless mutant, its addition should inhibit general protein synthesis as well as the induction of β -galactosidase. This prediction was confirmed by the experiment reported in Fig. 9, where the addition of galactose under starvation conditions inhibited the uptake of C^{14} -leucine into an acid-insoluble form. This inhibition was also reversed by the further addition of uracil. Moreover, the progressive onset of galactose inhibition of protein

FIG. 8. Reversal of galactose repression in gal E^- by uracil. Cells were pregrown in M95 glycerol medium containing 5×10^{-3} M fucose and 10^{-4} M uracil. Induction was carried out in the presence of IPTG and 10^{-4} M leucine, but in the absence of a metabolizable carbon source. (Note: leucine was present to measure rates of protein synthesis. Its presence does not influence the experimental results.) Symbols: $\circ$, control culture; $\square$, plus uracil; $\bullet$, plus galactose; $\blacksquare$, plus both uracil and galactose.

synthesis seen in Fig. 9 parallels the progressive repression of β -galactosidase seen in Fig. 7 and 8.

However, although uracil addition reversed the inhibition of protein synthesis by galactose in this experiment, it did not stimulate the differential rate of β -galactosidase synthesis. The appropriate calculations of β -galactosidase formed as a function of the overall rate of protein synthesis are shown in Fig. 10. It is apparent that uracil stimulated β -galactosidase formation only by increasing the general rate of protein synthesis, and not by overcoming the specific inhibitory effect of galactose on β -galactosidase formation.

We thus conclude that in the epimeraseless mutant the presence of galactose can inhibit the synthesis of other proteins as well as that of β -galactosidase, and that the mechanism of this effect is probably an inhibition of RNA synthesis following depletion of the UTP pool. When this general inhibition of protein synthesis is overcome by the further addition of uracil, a more specific effect of UDPgal accumulation on β -galactosidase formation remains, suggesting that UDPgal does indeed function in the regulation of this enzyme.

FIG. 9. Galactose inhibition of protein synthesis in epimeraseless cells. Cells of *gal E⁻* were pregrown in M95 glycerol medium containing 5×10^{-3} M fucose and 10^{-4} M uracil. C^{14} -leucine uptake in the presence of IPTG and the absence of a metabolizable carbon source was measured as described in Materials and Methods. Symbols are the same as in Fig. 8.

Growth inhibition of epimeraseless cells by galactose. Although depletion of the UTP pool appears to be a major effect of galactose on epimeraseless cells under starvation conditions, it does not appear to be the mechanism by which galactose inhibits the growth of this strain. With glycerol as carbon source, the addition of uracil had little effect in reversing the galactose inhibition of growth. This was true whether or not the cells were preadapted by growth in the presence of uracil. It seems likely then that high levels of UDPgal also inhibit some reaction, probably in carbohydrate metabolism, which is important during growth.

Role of UDPglu and its further metabolites. The other metabolic intermediate which may be considered as a component of the galactose pathway is UDPglu. A test of its possible participation in β -galactosidase repression was made with strain W4597, which lacks the enzyme uridine diphosphoglucose pyrophosphorylase. Cells lacking this enzyme are unable to make UDPglu from glucose-1-phosphate (glu-1-P) and UTP, and hence

UDPgal or any other metabolite uniquely derived from UDPglu. As Table 2 demonstrates, this mutant, as well as the epimeraseless mutant, remains sensitive to glucose repression, indicating that normal levels of the repressor are formed in these strains, although they cannot make UDPglu or UDPgal. It is therefore unlikely that UDPglu or any metabolite derived from it plays a significant role in the catabolite repression of β -galactosidase.

Strain 58-161. McFall and Mandelstam (15), in a study comparing the repression of several enzymes, previously came to the conclusion that exogenous galactose is a repressor of β -galactosid-

FIG. 10. Differential rate of β -galactosidase formation in epimeraseless cells. The data of Fig. 9 have been replotted to show the units of β -galactosidase formed as a function of the number of millimicromoles of C^{14} -leucine incorporated into acid-insoluble material. Symbols are the same as in Fig. 8.

TABLE 2. Catabolite repression in a *UDPglu⁻* strain^a

Carbon source	β -Galactosidase synthesis		
	W3110 (UDPglu ⁺)	W4597 (UDPglu ⁻)	W3805 (UDPgal ⁻)
Glucose.....	1.58	1.48	0.98
Xylose.....	2.09	2.03	1.85
Fructose.....	3.24	2.56	1.86
Glycerol.....	3.83	3.38	3.26
Lactate.....	4.39	4.51	3.50

^a Cultures were grown to between 2×10^8 and 4×10^8 cells per milliliter in each of the carbon sources in the presence of 5×10^{-4} M IPTG. β -Galactosidase was then assayed. Enzyme synthesis is expressed as enzyme units per unit absorbance of culture.

ase, and that it probably acts by forming UDPgal. Their conclusion was based upon finding that: (i) in a *met*⁺ revertant of *E. coli* K-12 strain 58-161 exogenous galactose was a more effective repressor than exogenous glucose when either was added to a culture growing in glycerol; (ii) a further mutant of this strain selected for loss of glucose repression of tryptophanase also lost glucose, but not galactose, repression of β -galactosidase; and (iii) kinase- and transferase-negative mutants of the series used in the present work were repressed by both galactose and glucose, whereas an epimerase-negative mutant was also repressed by galactose, but only weakly by glucose. Although the general conclusion reached in the earlier work of McFall and Mandelstam is similar to that arrived at here, there are some significant differences in the experimental results obtained in the two studies.

Item (i) is in disagreement with the results reported here for the wild-type strain of *E. coli* K-12 (W3110) and for *E. coli* B. Through the courtesy of Professor Mandelstam, I have been able to examine the inducibility of β -galactosidase in *met*⁺ 58-161 growing in single carbon sources and in the carbon source combinations employed by McFall and Mandelstam. No unusual repressive effect of galactose in this strain was found (Fig. 11). (Essentially identical results were obtained whether the carbon sources were present at 0.2%, as employed in the other studies reported here, or at 1%, as employed by McFall and Mandelstam.) The reason for the discrepancy between the results obtained in the two laboratories is not known at present. (Dr. McFall has recently suggested that the significant factor might be the cell density at the time of exposure to galactose.)

Item (ii) is somewhat difficult to evaluate, since we do not know whether the glucose-resistant mutant carries an altered regulatory system or an impaired glucose metabolism. It is not known whether it remains repressible by other carbon sources as well as by galactose, and whether galactose is also able to repress other enzymes sensitive to catabolite repression in this strain.

The principal facts of item (iii) are confirmed in the present experiments, although their interpretation is somewhat different. Galactose repression of kinase- and transferase-negative mutants is apparently the result of catabolite inhibition and only occurs if an additional carbon source is present. In the epimeraseless mutant, galactose represses by a complex set of mechanisms which include a general inhibition of protein synthesis, catabolite repression, and a specific effect of UDPgal on β -galactosidase formation. The first

FIG. 11. β -Galactosidase induction in strain 58-161. Cells were pregrown in M9S glycerol supplemented as described in Materials and Methods, and then transferred to fresh medium supplemented with IPTG and various carbon sources. Symbols: ○, glycerol alone; △, galactose alone; □, glucose alone; ●, glycerol plus galactose; ■, glucose plus galactose; ⊗, glucose plus glycerol.

of these can be overcome by addition of uracil, and the second by removal of any utilizable carbon source.

I did not, however, find any defect in the susceptibility of this strain to catabolite repression. As shown in Table 2, it remained equally sensitive with wild type to repression by glucose and other carbon sources.

DISCUSSION

Evidence was obtained for a specific role of UDPgal, but not galactose or gal-1-P, in β -galactosidase repression. Exogenous galactose was no more effective in repressing the enzyme during growth of prototrophic strains of *E. coli* K-12 and B than were other carbon sources. Galactose produced some effect when added under growth conditions to mutants blocked at the first or second steps in the galactose pathway. This effect presumably is derived from the ability of the galactosyl compounds to inhibit growth and from the general catabolite repression which develops during growth inhibition. The strongest

evidence against the identity of the endogenous repressor as galactose, or gal-1-P, came from experiments on the induction of β -galactosidase during carbon starvation in *gal K⁻* and *gal T⁻* cells. In this circumstance, when other carbohydrate intermediates do not accumulate, no repression of β -galactosidase was observed.

Experiments with the epimeraseless strain of *E. coli* growing under starvation conditions in the presence of uracil suggest that UDPgal may have a specific function in regulating the *lac* operon. These results do not indicate whether UDPgal acts directly, or whether its accumulation stimulates the formation of a transgalactosylation product which acts to regulate β -galactosidase formation.

It is significant that catabolite repression of β -galactosidase was normal in strains lacking UDPglu pyrophosphorylase or galactose epimerase, indicating that neither UDPglu nor its metabolic products act as the low molecular weight catabolite repressor. Thus, the present results argue against the notion that the phenomenon we call catabolite repression is the sum of a series of specific repressions; instead, it seems to represent a separate system of metabolic control. The regulation of the *lac* operon behaves as if it were sensitive to control by both a specific end-product repressor, which is UDPgal or a product thereof, and a generalized catabolite repressor, which can be formed from glucose in strains unable to make UDPglu or UDPgal.

ACKNOWLEDGMENTS

This investigation was supported by Public Health Service grant AI-03027 from the National Institute of Allergy and Infectious Diseases.

I am indebted to Norman Roggow, Francis Pacholec, and Susanne Griffiths for assistance in performing these experiments.

ADDENDUM

W. H. Beggs and P. Rogers (J. Bacteriol. **91**:1869, 1966) have reported experiments on galactose repression of the wild type and *K⁻ T⁻* strains used here. They concluded that it is not galactose itself, but a metabolic product thereof, which is active in repression. Their experiments are confirmed by the results reported here.

LITERATURE CITED

- ANDERSON, E. H. 1946. Growth requirements of virus-resistant mutants of *Escherichia coli* strain "B." Proc. Natl. Acad. Sci. U.S. **32**:120-128.
- BURSTEIN, C., M. COHN, A. KEPES, AND J. MONOD. 1965. Role du lactose et de ses produits métaboliques dans l'induction de l'opéron lactose chez *Escherichia coli*. Biochim. Biophys. Acta **95**:634-639.
- BUTTIN, G. 1963. Mécanismes régulateurs dans la biosynthèse des enzymes du métabolisme du galactose chez *Escherichia coli* K12. I. La biosynthèse induite de la galactokinase et l'induction simultanée de la séquence enzymatique. J. Mol. Biol. **7**:164-182.
- BUTTIN, G. 1963. Mécanismes régulateurs dans la biosynthèse des enzymes du métabolisme du galactose chez *Escherichia coli* K12. II. Le déterminisme génétique de la régulation. J. Mol. Biol. **7**:183-205.
- 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.
- ELBEIN, A. D., AND E. C. HEATH. 1965. The biosynthesis of cell wall lipopolysaccharide in *Escherichia coli*. I. The biochemical properties of a uridine diphosphate galactose 4-epimeraseless mutant. J. Biol. Chem. **240**:1919-1925.
- FUKASAWA, T., K. JOKURA, AND K. KURAHASHI. A new enzymic defect of galactose metabolism in *Escherichia coli* K12 mutants. Biochem. Biophys. Res. Commun. **7**:121-125.
- FUKASAWA, T., AND H. NIKAIIDO. 1961. Galactose-sensitive mutants of *Salmonella*. II. Bacteriolysis induced by galactose. Biochim. Biophys. Acta **48**:470-483.
- HERZENBERG, L. 1958. Studies on the induction of β -galactosidase in a cryptic strain of *Escherichia coli*. Biochim. Biophys. Acta **31**:525-538.
- HOFSTEN, B. 1961. Fluoro-D-galactosides as substrates and inducers of the β -galactosidase of *Escherichia coli*. Biochim. Biophys. Acta **48**:159-163.
- HORECKER, B. L., J. THOMAS, AND J. MONOD. 1960. Galactose transport in *Escherichia coli*. I. General properties as studied in a galactokinaseless mutant. J. Biol. Chem. **235**:1580-1590.
- KALCKAR, H. M., K. KURAHASHI, AND E. JORDAN. 1959. Hereditary defects in galactose metabolism in *Escherichia coli* mutants. I. Determination of enzymatic activities. Proc. Natl. Acad. Sci. U.S. **45**:1776-1785.
- KURAHASHI, K. 1957. Enzyme formation in galactose-negative mutants of *Escherichia coli*. Science **125**:114-116.
- KURAHASHI, K., AND A. J. WAHBA. 1958. Interference with growth of certain *Escherichia coli* mutants by galactose. Biochim. Biophys. Acta **30**:298-302.
- McFALL, E., AND J. MANDELSTAM. 1963. Specific metabolic repression of three induced enzymes in *Escherichia coli*. Biochem. J. **89**:391-398.
- MANDELSTAM, J. 1962. The repression of constitutive β -galactosidase in *Escherichia coli* by glucose and other carbon sources. Biochem. J. **82**:489-493.
- MONOD, J. 1956. Remarks on the mechanism of enzyme induction, p. 7. In O. H. Gaebler [ed.], Enzymes: units of biological structure and function. Academic Press, Inc., New York.
- MONOD, J., G. COHEN-BAZIRE, AND M. COHN.

1951. Sur la biosynthèse de la β -galactosidase (lactase) chez *Escherichia coli*. La spécificité de l'induction. *Biochim. Biophys. Acta* **7**:585-599.
19. MUELLER-HILL, B., H. V. RICKENBERG, AND K. WALLENFELS. 1964. Specificity of the induction of the enzymes of the *lac* operon in *Escherichia coli*. *J. Mol. Biol.* **10**:303-318.
20. NIKAIIDO, H. 1961. Galactose-sensitive mutants of *Salmonella*. I. Metabolism of galactose. *Biochim. Biophys. Acta* **48**:460-469.
21. PAIGEN, K. 1963. Changes in the inducibility of galactokinase and β -galactosidase during inhibition of growth in *Escherichia coli*. *Biochim. Biophys. Acta* **77**:318-328.
22. PAIGEN, K. 1966. A phenomenon of transient repression in *Escherichia coli*. *J. Bacteriol.* **91**:1201-1209.
23. PARDEE, A. B. 1957. An inducible mechanism for accumulation of melibiose in *Escherichia coli*. *J. Bacteriol.* **73**:376-385.
24. RICKENBERG, H. V., G. N. COHEN, G. BUTTIN, AND J. MONOD. 1956. La galactoside-permease d'*Escherichia coli*. *Ann. Inst. Pasteur* **91**:829-857.
25. SHEININ, R., AND B. F. CROCKER. 1961. The induced concurrent formation of α -galactosidase and β -galactosidase in *Escherichia coli* B. *Can. J. Biochem. Physiol.* **39**:63-72.
26. SPYRIDES, G. J., AND H. M. KALCKAR. 1960. Accumulation of uridine diphosphogalactose in a bacterial mutant defective in epimerase. *Biochem. Biophys. Res. Commun.* **3**:306-310.
27. WALLENFELS, K. 1960. Transgalactosylation. *Bull. Soc. Chim. Biol.* **42**:1715-1735.
28. WILLIAMS, B., AND K. PAIGEN. 1966. A group of compounds exhibiting paradoxical activity in the regulation of the *Lac* operon. *Biochem. Biophys. Res. Commun.* **24**:143-149.
29. YARMOLINSKY, M. B., H. WIESMEYER, H. M. KALCKAR, AND E. JORDAN. 1959. Hereditary defects in galactose metabolism in *Escherichia coli* mutants. II. Galactose-induced sensitivity. *Proc. Natl. Acad. Sci. U.S.A.* **45**:1786-1791.