AN INDUCIBLE MECHANISM FOR ACCUMULATION OF MELIBIOSE IN *ESCHERICHIA COLI*[1]

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Received for publication September 14, 1956

The idea that sugars enter living cells by some sort of "active transport system" has gathered support for some time (Rothstein, 1954; Davis, 1956). Permeability problems in sugar metabolism, for example in saccharide utilization (Doudoroff, 1951), speak for the importance of the problem to microbial growth.

In the present paper it is shown that cells of *Escherichia coli* which have not been previously exposed to the α-galactoside melibiose are largely impermeable to it; that an inducible enzyme-like system can be formed, upon exposure to suitable galactosides, which permits accumulation of melibiose and other galactosides; and that this system is distinct from α-galactosidase or β-galactosidase. Also, data are presented on the ability of various α-galactosides to support growth or to act as inducers of β-galactosidase of *E. coli*.

Recently, Monod (1956) has described an inducible system in *E. coli* capable of accumulating thiogalactosides; a more detailed report of that work[2] is to appear shortly (Rickenberg *et al.*, 1956). The system, galactoside-permease, appears to be the same as the one reported in the present communication.

MATERIALS AND METHODS

Escherichia coli, strains B or ML, was grown for 4 hr or more at 37 C with aeration by shaking. The medium contained 0.5 per cent glycerol in "minimal salts" [K_2HPO_4, 7 g; KH_2PO_4, 2 g; Na_3citrate$\cdot 5H_2O$, 0.5 g; $MgSO_4\cdot 7H_2O$, 0.1 g; and $(NH_4)_2SO_4$, 1 g; in water, 1 L]. The concentration of bacteria was determined by optical density measurements with the Beckman spectrophotometer, at 570 mμ. An optical density of 0.1 was equal to 2×10^8 bacteria per ml.

The amounts of protein, fructose, or reducing sugar were determined by the Folin (Lowry *et al.*, 1951), Roe, and Folin-Malmros (Umbreit *et al.*, 1949) methods, respectively. Nonreducing derivatives of sugars, such as melibiitol, were hydrolyzed 15 min at 100 C in 0.5 N HCl to release the reducing groups before estimation. Radioactive sulfur was measured with an end-window Geiger-Mueller counter.

Chromatography of sugars was performed according to Hough *et al.* (1950) and the spots were detected with *p*-anisidine spray. The sugars also were chromatographed as their N-(1-naphthyl)-ethylene diamine derivatives by the method of Wadman *et al.* (1954).

The activity of β-D-galactosidase was measured with toluene-treated bacteria by incubation for 15 min at 37 C of 1 ml of bacterial suspension containing about 2×10^8 cells with 1.5 ml of 0.1 M sodium phosphate, pH 7.0, containing 1 mg of *o*-nitrophenyl-β-D-galactoside (NPG), addition of 2.5 ml of acetone, and determination of blue light absorption with the Klett colorimeter (Koppel *et al.*, 1953). The activity of α-D-galactosidase was measured by determining the rate of melibiose oxidation by intact, washed bacteria in the presence of 10 μg per ml of "chloromycetin," using the Warburg respirometer. Chloromycetin at this concentration prevented increase of enzyme activity during the assay (Koppel *et al.* 1953).

All concentrations represent final concentrations. All sugars used in this work were of the D configuration. Melibiose (6-O-α-D-galactopyranosyl-D-glucose), sucrose, lactose, galactose, and raffinose were recrystallized from commercial products. Epimelibiose (6-O-α-D-galactopyranosyl-D-mannose) was kindly provided by Dr. R. L. Whistler. Isomaltose (6-O-α-D-glucopyranosyl-D-glucose) and galactosylglycerol (2-O-α-D-

[1] This work was aided by grants from the Lederle Laboratories Division of the American Cyanamid Company and The Rockefeller Foundation.

[2] The author is indebted to Dr. J. Monod for a copy of the manuscript prior to publication.

galactopyranosyl glycerol) and inulin were furnished by Dr. E. W. Putman, and galactinol (1-O-α-D-galactopyranosyl mesoinositol) and melibiitol (6-O-α-D-galactopyranosyl D-glucitol) by Dr. C. E. Ballou. Methyl α- and β-D-galactosides were synthesized by Mr. George Rushizky. Ethyl-β-thiogalactoside was synthesized by Mr. George H. Hubert (Rickenberg *et al.*, 1956), DL-β-2-thienlyalanine was obtained from the Arapahoe Chemical Co., and chloromycetin was a gift from Parke, Davis and Co.

RESULTS

Elimination of the lag in induction of β-galactosidase. Melibiose is a good inducer of β-galactosidase in *E. coli* (Monod *et al.*, 1951), but the enzyme appears after a lag of some 20 min in *E. coli* strain B grown on glycerol (figure 1*a*, curve A). When the bacteria were grown in the presence of galactinol, β-galactosidase was formed with no lag upon addition of melibiose (curves *C* and *D*). Therefore, an initial process, necessary before enzyme formation is apparent, must have been separately induced by galactinol. An investigation, presented below, of this initial process led to the conclusion that an inducible mechanism was formed which permitted melibiose to enter the cell and be accumulated. This mechanism will be called the "accumulation mechanism" in the remainder of the article.

Exposure to galactinol must also have induced an enzyme that attacks melibiose, for it is seen (figure 1*b*, curve *C*) that the bacteria grown in the presence of galactinol later grew rapidly on melibiose as the sole carbon source. In contrast, bacteria grown on glycerol subsequently grew very slowly on melibiose; but they were able to form β-galactosidase after a lag of more than 20 min (curve *B*), as noted previously (Pardee 1955).

Glycerol actually decreased the initial rate of enzyme formation by the galactinol-grown bacteria, relative to growth or relative to time of induction (curves *C* and *D*), although there was no period during which enzyme formation failed to occur. This result might be explained in terms of a competition for nutrients (such as amino acids or nucleotides) between the enzyme-forming mechanisms involved in making β-galactosidase and those involved in forming the glycerol-oxidizing system (Pardee, 1955; Spiegelman *et al.*, 1955).

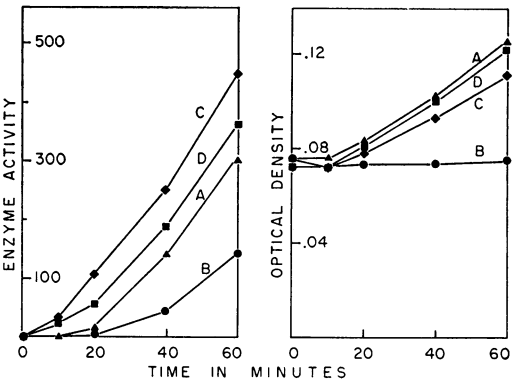

Figure 1a (left), 1b (right). Effect of growth in the presence of galactinol on subsequent induction of β-galactosidase. *Escherichia coli* strain B were grown in the presence or absence of 1 mg per ml of galactinol in minimal medium for 3 hr to a concentration of about 2×10^8 cells per ml. The bacteria were washed by centrifugation and resuspended in fresh media lacking galactinol, with 2 mg per ml of melibiose as inducer and with or without 5 mg per ml of glycerol as a supplementary energy source. Turbidity and β-galactosidase (μg *o*-nitrophenyl-β-D-galactoside (NPG) hydrolyzed in 15 min per ml of culture) were determined in aliquots at intervals over a 1-hr period. *A.* ▲ = Grown without galactinol; induction in presence of glycerol. *B.* ● = Grown without galactinol; induction in absence of glycerol. *C.* ◆ = Grown in presence of galactinol; induction in absence of glycerol. *D.* ■ = Grown in presence of galactinol; induction in presence of glycerol.

Comparison of nutrition and β-galactosidase induction by α-galactosides in E. coli strains B and ML. The above results were obtained with *E. coli* strain B, and since the initial observations on induction of β-galactosidase by melibiose were made with strain ML (Monod *et al.*, 1951) some comparison between the two strains seemed worthwhile. Furthermore, it was necessary to obtain information regarding the ability of the strains to grow and to form galactosidases on exposure to various galactosides, in order to interpret later data. Table 1 shows that the strain ML used did not grow on any α-galactoside tested or on galactose as sole carbon source in 40 hr. By contrast, strain B could use most of these compounds as a sole carbon source. Strain ML was unable to oxidize melibiose, as shown by

TABLE 1

Effects of galactosides on Escherichia coli

Compound	Growth		β-Galactosidase Inducer		Lag Period	
	B	ML	B	ML	B	ML
Melibiose	++	−	++	++	+++	+++
Epimelibiose	++	−	−	−	+++	−
Galactinol	+	−	−	−	+	−
α-2-Glyceryl galactoside	++	−	−	−	+	−
Melibiitol	++	−	−	−	++	−
Me-α-galactoside	+	−	(+)	++	+++	+++
Raffinose	−	−	−	−	−	−
Galactose	++	−	−	+	−	+++
Lactose	++	++	+++	+++	+++	+++
Me-β-galactoside	−	−	+++	+++	+++	+++
Et-β-thiogalactoside		−		+++		+++
Isomaltose	−	−	−	−	−	−

Experimental details are given in the text. For growth, ++ denotes strong turbidity within 18 hr, + denotes strong turbidity within 40 hr, and − denotes no turbidity within 48 hr from an inoculum of 10^5 *Escherichia coli*. For β-galactosidase induction, +++ denotes ability of 10^8 bacteria to hydrolyze more than 100 μg *o*-nitrophenyl-β-D-galactoside (NPG) per 15 min after 1 hr exposure to the designated compound, ++ indicates a rate of about 50 μg per 15 min, and (+) means a trace of activity. For reduction of the lag period, +++ means that bacteria exposed to the compound for 1 hr showed no lag upon induction by melibiose, ++ means delayed initial rate, + means definitely delayed initial rate, and − means lag was like that of the control.

experiments with the Warburg respirometer, and therefore must lack the ability to form α-galactosidase.

Compounds structurally similar to melibiose were tested as inducers in order to learn more about the specificity of β-galactosidase induction by α-galactosides. These experiments were performed by addition of the α-galactoside (0.5 mg per ml) for 1 hr to an exponentially growing culture of *E. coli* containing 5×10^8 bacteria per ml, removal of the medium by centrifugation, and determination of β-galactosidase activity in an aliquot of the resuspended bacteria. The results show that melibiose was the only α-galactoside at this concentration that acted as a strong inducer for strain B (table 1). Melibiose, Me-α-galactoside, and galactose were fairly good inducers for strain ML within 1 hr, but other α-galactosides were inactive. The inducing ability of melibiose for β-galactosidase therefore depends on specificity in the aglycone portion of the molecule, as well as in the galactosyl portion. The carbon source on which the bacteria were grown influenced ability of the galactosides to act as inducers: maltose was slightly inhibitory relative to glycerol.

Effects of various compounds on subsequent induction by melibiose. Experiments were performed to determine whether various α-galactosides and related compounds were able to abolish the lag in induction by melibiose of β-galactosidase. The bacteria were grown for 1 hr in minimal medium plus 0.5 mg per ml of the compound. The cells were centrifuged; then growth was resumed in fresh media containing melibiose, 1 mg per ml, and glycerol, 5 mg per ml, for periods up to an additional ½ hour before enzyme assay. This transfer into fresh media was necessary since some of the compounds, at least melibiitol and glycerylgalactoside, were able to inhibit induction by melibiose (see below). Effects on the lag periods of β-galactosidase formation are listed in table 1. Many α-galactosides shortened or eliminated the lag with strain B while only galactose, melibiose, or Me-α-galactoside did so for strain ML. Thus, the two strains differ markedly in their responses to these sugars. It may be noted that galactinol, used to obtain figure 1, was not as effective in reducing the lag as were most of the compounds. Also, the β-galactosides were highly effective in reducing the lag.

Certain α-galactosides not only eliminated the lag without inducing β-galactosidase, but actually inhibited the formation of β-galactosidase by

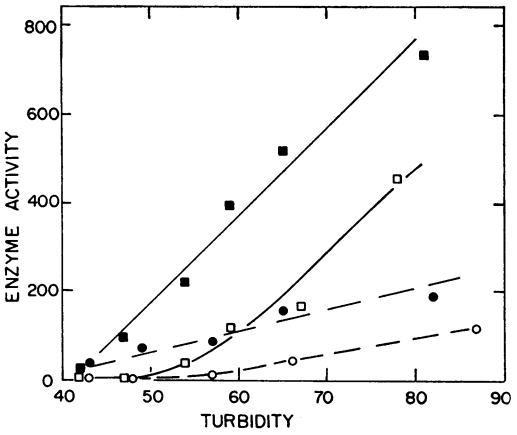

Figure 2. Inhibition of formation of β-galactosidase. *Escherichia coli* strain B was grown in minimal medium to a concentration of 3×10^8 bacteria per ml. During the last hour, half the culture was grown in the presence of 500 μg per ml methyl-α-galactoside. The cells were centrifuged, and resuspended in fresh minimal medium, in the presence and absence of 1.3 μg per ml β-2-thienylalanine (βTA). Melibiose (100 μg per ml) was added, and at subsequent times aliquots were removed for determination of β-galactosidase. Activities are given as μg *o*-nitrophenyl-β-D-galactoside (NPG) hydrolyzed per 15 min per ml of culture, and are plotted against turbidity. ■ = growth with methyl-α-galactoside; induction without βTA. ● = growth with methyl-α-galactoside; induction with βTA. □ = growth without methyl-α-galactoside; induction without βTA. ○ = growth without methyl-α-galactoside; induction with βTA.

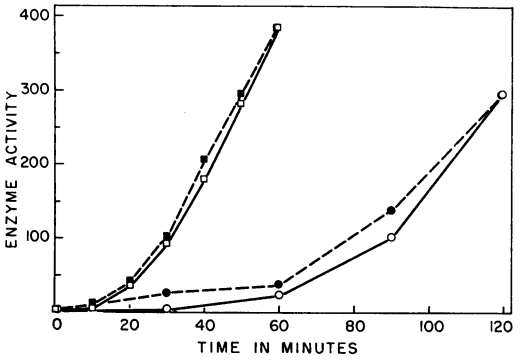

Figure 3. Induced formation of α- and β-galactosidases in the presence or absence of β-2-thienylalanine (βTA). Melibiose (1 mg per ml) and βTA (1 μg per ml) were added to *Escherichia coli* strain B growing in minimal medium. Aliquots were removed at various times and assayed for α- or β-galactosidase. Enzyme activities are in arbitrary units. ■ = α-Galactosidase formed in the absence of βTA. □ = β-Galactosidase formed in the absence of βTA. ● = α-Galactosidase formed in the presence of βTA. ○ = β-Galactosidase formed in the presence of βTA.

melibiose. Exposure of exponentially growing *E. coli* strain B to melibiitol (0.5 mg per ml) for 1 hr largely eliminated the lag upon subsequent induction by melibiose in fresh medium but did not cause formation of β-galactosidase. With these bacteria, 0.5 mg per ml of melibiitol showed an 85 per cent inhibition of β-galactosidase formation by 0.5 mg per ml melibiose. It cannot be decided from this experiment whether the effect is due to inhibition of penetration of melibiose into the cell or inhibition of the action of intracellular melibiose in some later process of induction.

Various α-galactosides in the presence of chloromycetin, 10 μg per ml, were ineffective in causing a reduction in the lag period on subsequent induction of β-galactosidase by melibiose.

Inhibition by β-2-thienylalanine (βTA). The idea that induction by melibiose occurs in two steps would be supported if it were possible specifically to inhibit the individual steps. Such an effect was found with the amino acid analogue βTA which has been found to inhibit β-galactosidase formation preferentially to protein synthesis in *E. coli* strain B (Pardee and Prestidge, 1955). At 1.3 μg per ml this inhibitor reduced the growth rate by a factor of two, and it increased the time lag of β-galactosidase formation by a factor of at least four and the rate of enzyme formation was reduced to about 1/7 (figure 2). Thus the lag and the subsequent rate of enzyme formation are altered to different extents. A greater difference was observed with bacteria which had been grown in the presence of methyl-α-galactoside. In these bacteria the lag had been abolished and was not restored or otherwise affected by βTA; however the rate of enzyme formation was still inhibited to the same extent as in the control. Inhibition by βTA was dependent on the inducer: inhibition of β-galactosidase formation was much less when lactose was used as inducer than with melibiose and was greater with 0.1 mg per ml than with 1 mg per ml lactose.

The effect of βTA on induction of β-galactosidase by melibiose was compared with its effect on α-galactosidase formation (figure 3). It is seen

that not only were the lag periods similar for the two enzymes in the absence of inhibitor, but also this lag period was extended similarly by 1 μg per ml βTA and the rate of enzyme formation was inhibited to the same extent. These results are in contrast with the weaker effects of the inhibitor on other processes: it seems very likely that reactions common to formation of both enzymes are affected.

Permeability of E. Coli to melibiose. The above results demonstrate that at least two processes are involved in the induction of β-galactosidase by melibiose. The question is immediately raised as to the nature of the first step. Dependence on the presence of α-galactosides and inhibition by chloromycetin or by βTA, an amino acid analogue, are strongly indicative of induced synthesis of some active protein (Spiegelman *et al.*, 1955). A most likely possibility is that formation of a system for introduction of melibiose into the bacterial cell may be involved. Two aspects of this possibility are open to test. The following questions were asked: first, whether cells of *E. coli* are freely permeable to melibiose; second, whether *E. coli* possesses a mechanism to accumulate the sugar in the cells at a higher concentration than in the medium. Also, attempts were made to learn whether the mechanism had the same properties in strains B and ML, and whether the sugar added to the medium could be recovered unchanged from the bacterial cells.

Passive permeability. The ability of *E. coli* to take up melibiose and other compounds from the medium was determined by two independent methods (Bolton *et al.*, 1955): by measurement of the amount of the compound removed from the medium, and by determination of the amount of the compound found within the bacteria. A typical experiment is as follows:

E. coli strain B were grown aerobically in 1.2 L minimal medium at 37 C to a concentration of 10^9 bacteria per ml. The culture was chilled and the bacteria were centrifuged into one pellet (weight 1.39 g). The pellet was suspended in 1.80 ml of cold medium containing 10 μg per ml of chloromycetin (to prevent subsequent induction of an accumulation mechanism). Aliquots (0.50 ml) were placed in 50-ml plastic centrifuge tubes and brought to either 0 or 25 C. Then 0.05 ml of medium containing melibiose, 10 mg per ml; sucrose, 10 mg per ml; uracil, 10 mg per ml; and $S^{35}O_4^=$ (2×10^6 counts per min per ml) were added to each aliquot. Uracil, sulfate, and sucrose (or inulin) were added to decide, by comparison, whether melibiose behaved like a compound to which the bacteria were permeable or nonpermeable. The samples were aerated by shaking for 5 min, chilled, and centrifuged for 10 min at 8,000 $\times$ G and the supernatants were collected. The plastic tubes were wiped carefully and 0.5 ml of 5 per cent trichloracetic acid (TCA) was mixed with each precipitate. After 30 min at 0 C the extracts were collected by centrifugation. Extracts and supernatants were analyzed for the added compounds (melibiose as reducing sugar, sucrose by the Roe test, sulfate by counting, and uracil by optical density at 260 mμ). Samples lacking bacteria, without added compounds, and with the compounds at half the above concentrations, were run simultaneously.

Results of experiments on uptake of various compounds are summarized in table 2. First, it may be seen that results based on the amounts remaining in supernatants are generally in agreement with those based on amounts of compounds found in the bacteria. Slightly higher values by the latter measurements may reflect metabolism. The bacteria seem freely permeable to pyrimidines (about 90 per cent of the pellet volume) in all cases. Thirty to 40 per cent of the pellet volume is available to sucrose, and slightly less (20 to 30 per cent) to inulin or sulfate. These results may be interpreted in terms of the model of Conway and Downey (1950): the intracellular space (20 per cent) contains sulfate and inulin, sucrose can enter the cell-wall space (10 per cent) as well, and the entire water volume of the pellet (80 per cent) is available to pyrimidines. The results with sulfate are in approximate agreement with the most recent results of Bolton *et al.* (1955) who found slight penetration.

The ability of the bacteria to take up melibiose depended on the conditions under which they had been grown and were tested. *E. coli* strain B which had been grown in the presence of melibiose were subsequently able to cause a considerable fraction of this sugar to disappear at 25 C (experiment I) but not at 0 C (experiment II). However, a corresponding amount of reducing sugar was not found in the bacterial extract. It is clear that melibiose was metabolized at 25 C, but whether or not it entered the cell cannot be determined, since it may have been split at the bacterial surface. No special means was used to supply air to these dense suspensions. *E. coli* strain B which had been grown without melibiose took up no

TABLE 2

Fraction of pellet volume of Escherichia coli available to various compounds

Experiment	I		II		III		IV†		V		VI	
Strain	B		B		B		B		ML		ML	
Medium*	+		+		−		−		−		+	
Temperature	25		0		25		0		25		25	
Aeration	−		−		+		−		−		+	
Melibiose	500	40	46	28	40	27	25	28	37	28	71	61
Sucrose	36	27	39	30	45	35	24	33	40	32		
Inulin									28	25	10	30
$S^{35}O_4^=$	19	20	21	20			12	19	33	20	37	47
Uracil	89	50	86	89					100	73		
Thymin	108	75			124	87	109	93	105	93		
Orotate											91	52

Experimental details are given in the text. Values are per cent of pellet volume available to the compound. Left-hand numbers are determined by measurements of removal from medium and right-hand ones from amounts found in bacteria.

* The + sign means that the bacteria were grown in the presence of melibiose; − means that they were grown in minimal medium.

† The uptakes of sucrose and melibiose were determined separately in this experiment.

more melibiose than sucrose from the medium, either at 25 C with "aeration" (experiment III) or at 0 C (experiment IV).

E. coli strain ML grown in the absence of melibiose did not subsequently take up melibiose at 25 C (experiment V). This experiment shows that the bacteria were not *passively* permeable to the sugar. Growth of *E. coli* strain ML in the presence of melibiose permitted the entry into the bacteria at 25 C (but not at 0 C) of somewhat more melibiose than inulin or sulfate (experiment VI). However, the difference, though definitely greater than found in experiment V, was not striking. Under these experimental conditions, it is likely that the induced bacteria cannot take in melibiose efficiently by an active mechanism in view of the fact that they are not in an optimal environment (high cell concentration, low O_2 tension), nor good physiological condition owing to extensive pretreatment, and because there is considerable error inherent in the entire procedure (because of high analytical blanks and attempts to measure a small difference between large numbers). The results presented below show that the conditions were far from optimal in regard to ability to accumulate sugars, therefore experiments in dense bacterial suspensions were not pursued further.

Accumulation of melibiose. The striking observation made in the above experiments was that a higher concentration of reducing sugar was found in *E. coli* strain ML than was put into the medium from which the cells were harvested. This means that there was an active accumulation of sugar during growth. The ability of *E. coli* strain ML to accumulate melibiose was tested by permitting the bacteria to grow to a concentration of 10^9 cells per ml in various media, then adding chloromycetin, 10 μg per ml, and melibiose, 100 μg per ml, and continuing incubation for various times. The bacteria were harvested; the pellet was weighed, then extracted with 5 per cent TCA; and extracts and medium were analyzed for reducing sugars. Results (table 3) showed very definitely that *E. coli* strain ML, which had been grown on melibiose, accumulated reducing sugar, to a concentration 20 times that in the medium. Bacteria grown in the presence of galactose also accumulated reducing sugar, but subsequently were able to accumulate more sugar when exposed to melibiose in the presence of chloromycetin. They had set up an accumulation mechanism which was lacking in the bacteria grown on minimal medium. The data show also that the formation of the accumulation mechanism is sensitive to chloromycetin but the accumulation process is not. *E. coli* strain B did not accumulate reducing sugars under any circumstances; however, these bacteria grown in the presence of melibiose or galac-

TABLE 3

Accumulation of reducing sugars by Escherichia coli strain ML

Inducer		Substrate		Intracellular Sugar
	min		*min*	*μg/ml*
	240		45	0
Melibiose	240		45	2,500
Galactose	240		45	650
	240	Melibiose	45	4
Melibiose	240	Melibiose	45	2,800
Galactose	240	Melibiose	45	2,300
	90		80	0
	90	Melibiose	80	6
Galactose	90		0	1,400
Galactose	90	Melibiose	20	2,200
Galactose	90	Melibiose	40	2,300
Galactose	90	Melibiose	60	2,300
Galactose	90	Melibiose	80	1,900

E. coli strain ML in the exponential stage of growth were exposed to the inducer at a concentration of 100 μg per ml for the designated time; then 10 μg per ml "chloromycetin" and the substrate at 100 μg per ml were added and the cultures were shaken for the designated time at 37 C. The cells were centrifuged and extracted, and an aliquot was assayed for reducing sugar. Intracellular sugar is given in μg per ml (calculated as melibiose) of reducing sugar in excess of the amount found in bacteria not exposed to any sugar.

tinol rapidly removed melibiose from the medium in the presence of chloromycetin. They must have metabolized melibiose as rapidly as it entered the cells.

Some of the properties of the accumulation system are shown in figure 4. A lag of about 30 min was observed before reducing sugar began to accumulate in the bacteria after exposure to melibiose. The correspondence between this lag and the one required before β-galactosidase formation commenced indicates that the accumulation mechanism must be set up before the enzyme formation commences. The effect of pretreatment (15 min with ethyl-β-thiogalactoside, 40 μg per ml) is also seen in the figure. There was immediate uptake of reducing sugar upon exposure to melibiose and also immediate continuation of β-galactosidase formation. The initial high reducing sugar value in these cells must be attributed to melibiose uptake during the period of chilling and centrifuging, since ethyl-β-thiogalactoside does

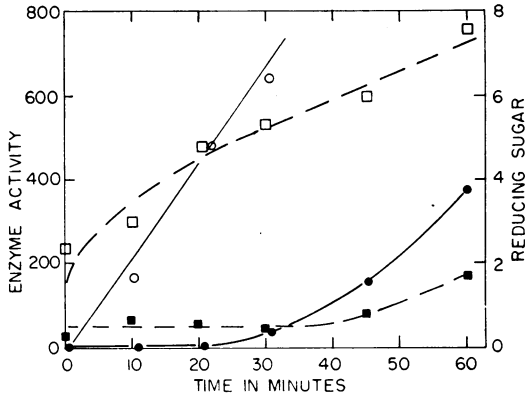

Figure 4. Reducing sugar accumulation and enzyme formation. *Escherichia coli* strain ML was grown on minimal medium to a concentration of 5 × 10^8 bacteria per ml. During the last 15 min, a portion of the culture was grown in the presence of ethyl-β-thiogalactoside, 40 μg per ml. The cells were centrifuged and resuspended in fresh minimal medium in the presence and absence of 100 μg per ml melibiose, and at subsequent times aliquots were removed and assayed for reducing sugar and β-galactosidase. Reducing sugar values are given as μg melibiose inside the bacteria per ml of culture. (Correction is made for the reducing sugar found in the culture lacking melibiose.) Enzyme activity is given as μg *o*-nitrophenyl-β-D-galactoside (NPG) hydrolyzed per 15 min per ml of culture.

β-Galactosidase: ● = Growth without ethyl-β-thiogalactoside. ○ = Growth with ethyl-β-thiogalactoside (initial activity subtracted).

Reducing sugar: ■ = Growth without ethyl-β-thiogalactoside. □ = Growth with ethyl-β-thiogalactoside.

not give rise to reducing sugar in this strain of *E. coli*.

Nature of the sugar accumulated by E. coli strain ML. Extracts (prepared with boiling water or cold 3 per cent $HClO_4$) of *E. coli* strain ML which had accumulated reducing sugars were chromatographed by two different techniques. By both methods, the only intense spot was located at the site for disaccharides. This spot was not found in the controls grown in minimal medium, but both samples contained a number of fainter spots, some corresponding to hexoses and pentoses. The sugar that was accumulated was thus principally a disaccharide, probably either melibiose or a labile derivative which decomposed upon preparation for chromatography.

Extracts of *E. coli* strain ML exposed to meli-

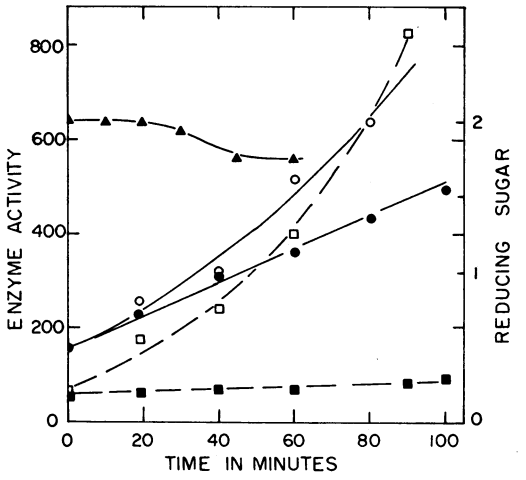

Figure 5. β-Galactosidase accumulation in the absence of added inducer. *Escherichia coli* strain ML was grown in minimal medium to a concentration of 4 × 10^8 bacteria per ml. During the last hour portions were grown in the presence of 10 μg per ml galactose or 100 μg per ml melibiose. The cells were centrifuged and resuspended in fresh minimal medium in the presence and absence of inducers as listed below. One sample of melibiose-grown bacteria was exposed to an amount of medium from the galactose-grown bacteria in excess of that carried over by the pellet of packed cells. Aliquots were removed at subsequent times for assay of reducing sugar (in one case) and for β-galactosidase. Units of enzyme activity and reducing sugar are as in figure 4.

β-galactosidase: ○ = Growth with galactose; induction with galactose. ● = Growth with galactose; induction without galactose. □ = Growth with melibiose; induction with melibiose. ■ = Growth with melibiose; induction without melibiose, but with 0.2 ml medium from galactose-grown bacteria.

Reducing sugar: ▲ = Growth with galactose; induction without galactose.

biose did not stimulate β-galactosidase induction any more than did extracts of bacteria grown on glycerol alone; therefore, no evidence for formation of a new inducer from melibiose was provided by these data.

Extracts of *E. coli* strain ML grown in the presence of galactose were chromatographed in the same manner as above. The principal material was found to have an R_f like that of galactose, and not to move like samples of glucose-6-phosphate or lactose.

Properties of the accumulation mechanism. The maximum amount of melibiose was accumulated within 20 min by *E. coli* strain ML previously grown on galactose (table 3). The amount of reducing sugar accumulated depended on the external concentration of melibiose in the manner described by Rickenberg *et al.* (1956). The concentration in the medium required for half saturation of the bacteria was 10^{-3} M melibiose and the "capacity" (maximum attainable concentration in the bacteria) was 0.8 per cent of the dry weight. The concentration of melibiose that induced half the maximum amount of β-galactosidase in 100 min was much lower, 2 × 10^{-4} M. Pretreatment with ethyl-β-thiogalactoside permitted accumulation of much more melibiose (5 per cent of the cell dry weight from 100 μg per ml melibiose). The concentration of galactose that saturated the bacteria was less than 3 × 10^{-5} M and the capacity was 0.8 per cent. The concentration in the bacteria was over 200 times that in the medium.

TABLE 4

Specificity of induction and transport

Inducer		Substrate	Intracellular Sugar
	min		*μg/ml*
None	180	Melibiose	210
	180	Me-β-galactoside	480
Melibiose	180	None	110
	180	Melibiose	1,260
	180	Me-β-galactoside	3,420
Galactose	180	None	2,220
	180	Melibiose	4,150
	180	Me-β-galactoside	3,330
Me-β-galactoside	20	None	2,300
	20	Melibiose	4,000
Ethyl - β - thio-galactoside*	60	None	600
	60	Melibiose	6,400

Escherichia coli strain ML were treated as for the experiment of table 3, but they were separated from the inducer (except in the case of Me-β-galactoside) before being added to the substrate and chloromycetin in the fresh medium. They were shaken with the substrate for 30 min. Intracellular sugar is given in μg per ml (as melibiose) above the amount found in bacteria not exposed to any sugar.

*40 μg per ml of this compound was used.

Leakage of sugars from the bacteria. E. coli strain ML were permitted to accumulate melibiose, and were then centrifuged and resuspended in fresh medium to determine the rate at which the reducing sugar escaped from the cells. All melibiose escaped from the bacteria within 10 min at 37 C. About one-third of the melibiose escaped in 30 min at 0 C. Galactose, by contrast, did not escape rapidly: little leakage was observed in 60 min at 37 C. These bacteria continued to form β-galactosidase in the absence of extracellular galactose, an effect similar to that described by Pollock (1953) for penicillinase (figure 5).

Specificity of induction of the accumulation system. As has already been noted, galactose is able to induce the system for accumulation of melibiose in *E. coli* strain ML. Galactose also was capable of inducing a system for accumulation of Me-β-galactoside (as a reducing derivative) when the bacteria were exposed to this glycoside (table 4). Melibiose also made these accumulations possible. Conversely, Me-β-D-galactoside permitted accumulation of melibiose; however, this result might be attributed to the galactose formed upon removal of the methyl group. Ethyl-β-thiogalactoside was an especially powerful inducer of melibiose accumulation.

DISCUSSION

With the appearance of the definitive publication of Rickenberg *et al.* (1956), the present work becomes of interest as an independent confirmation and an extension of the observations made on the galactoside-permease system. It is certain that the two systems are the same, since ethyl-β-thiogalactoside permitted accumulation of melibiose. Rickenberg *et al.* report that accumulation of Me-β-thiogalactoside by *E. coli* is inhibited by lactose or melibiose; this result indicates an absence of α-β specificity. As reported above (table 4), galactose induced the ability for accumulation of either melibiose or Me-β-galactoside in *E. coli* strain ML; also, prior treatment of either strain of *E. coli* with β-galactosides abolished the lag in induction by melibiose (table 1). Therefore the simplest assumption is that one system is induced for accumulation of both- α and β-galactosides. The galactoside permease system in *E. coli* strain ML can thus accumulate galactose, melibiose, and β-galactosides as well as β-thiogalactosides. The specificity of *E. coli* strain B is different from that of strain ML, as judged from table 1.

It was shown in this paper that almost the entire volume of *E. coli* is impermeable to melibiose and other galactosides before induction. Exposure of the bacteria to various galactosides permitted accumulation of poorly utilized galactosides (melibiose, Me-β-galactoside, or galactose) in *E. coli* strain ML, or metabolism of utilizable galactosides (α-galactosides and galactose) in *E. coli* strain B.

The induction of galactoside-permease in *E. coli* strain B was accomplished by various α-galactosides without induction of β-galactosidase. Galactoside-permease formation was less inhibited than β-galactosidase formation by β-2-thienylalanine. These results show that the permease and the galactosidase are not the same. Also, inhibition by the amino acid analogue, βTA, and by chloromycetin strongly suggest that formation of enzyme-like materials are involved.

Two sorts of results are presented relevant to the ability of α-galactosides to induce β-galactosidase formation. First, it appears evident that the lag in induction of β-galactosidase by melibiose represents the time required to set up the galactoside permease system and to accumulate melibiose in the cell. Second, two of seven α-galactosides tested, and also galactose, acted as inducers of β-galactosidase in *E. coli* strain ML. These results show a definite specificity for the aglycone, but the induction cannot be considered as exclusively a property of β-galactosides. One may make the hypothesis that there is no α-β specificity; rather that steric hindrance between the aglycone of most α-galactosides and some surface (permease, another enzyme, or β-galactosidase-forming system) can prevent the inducer action.

ACKNOWLEDGMENTS

We wish to thank Mr. Thomas Briggs for assistance in the early parts of this investigation, and Drs. M. Doudoroff, G. Cohen-Bazire, C. E. Ballou, and E. W. Putman for helpful discussions. We are indebted to Drs. C. E. Ballou, R. L. Whistler, and E. W. Putman for providing compounds, and to Dr. G. S. Stent for radioactive sulfate.

SUMMARY

Escherichia coli strains B or ML not previously exposed to melibiose do not permit entry

of this sugar into most of the cell; nor do the bacteria permit entry of sucrose, inulin, or sulfate, but they are freely permeable to uracil and thymin.

After exposure to either galactose, melibiose, Me-β-galactoside, or ethyl-β-thiogalactoside, *E. coli* strain ML can concentrate any of these sugars at an internal concentration of at least 20 times the level in the medium. Sugars can be recovered unchanged from the bacteria.

Data on properties of the accumulation mechanism lead to the conclusion that it is an inducible, enzyme-like system. It appears identical to the recently described galactoside-permease, and is not the same as α-galactosidase or β-galactosidase.

Melibiose, Me-α-galactoside, and galactose were able to induce β-galactosidase, but five other α-galactosides could not. Most of the α-galactosides tested could induce the permease of *E. coli* strain B but not in strain ML. The lag in induction of β-galactosidase by melibiose is attributed to the time required for penetration of the sugar into the bacterial cell.

E. coli strain ML grown in the presence of galactose was subsequently able to form β-galactosidase as rapidly in the absence of galactose as in its presence. This effect is attributed to the slow escape of the accumulated galactose from inside the bacteria.

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