BBA 75074

INHIBITION OF β-GALACTOSIDE TRANSPORT BY SUBSTRATES OF THE GLUCOSE TRANSPORT SYSTEM IN *ESCHERICHIA COLI*

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(Received April 19th, 1967)

SUMMARY

1. The uptake by *Escherichia coli* (ML 308) of thiomethyl-β-galactoside and *o*-nitrophenyl-β-galactoside was inhibited by glucose, α-methylglucoside and other compounds which are substrates for the glucose transport system. This inhibition of β-galactoside transport was observed only in glucose-grown cells. Such cells possessed a five-fold greater α-methylglucoside transport capacity (v_{max}) than cells grown on other carbon sources, the affinity for uptake (K_t) being the same in both types of cells. Growth of cells on carbon sources (other than glucose) which resulted in levels of catabolite repression and glucokinase equivalent to those in glucose-grown cells did not render the cells susceptible to inhibition. Although cells grown on glucose accumulated more α-methylglucoside and α-methylglucoside phosphate than cells grown on other carbon sources at a given extracellular concentration of α-methylglucoside these intracellular pools could be made equally large in casein amino acid-grown cells by increasing the extracellular concentration of α-methylglucoside. Thus, a high intracellular level of α-methylglucoside and its phosphorylated derivative is not a sufficient condition for the production of cells sensitive to α-methylglucoside.

2. β-Galactosides did not inhibit the uptake of α-methylglucoside, and α-methylglucoside was unable to cause "counterflow" of thiomethyl-β-galactoside. Such experiments indicate the lack of reciprocity between galactoside and glucose transport systems and put severe restrictions on the "common carrier" or *transporter* hypothesis. The entrance of thiomethyl-β-galactoside and *o*-nitrophenyl-β-galactoside into metabolically poisoned cells was inhibited by α-methylglucoside indicating that energy coupling is not involved in the inhibitory phenomenon.

3. It is suggested that the product of the "y" gene is the membrane carrier *per se* and that substrate–carrier complex requires a factor which is common to many carbohydrate transport systems and limited in amount. Thus, α-methylglucoside–carrier complex when present in large amounts combines with a significant fraction of the common factor making it unavailable for the β-galactoside transport system.

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INTRODUCTION

The active transport of sugars into bacteria is inhibited by a variety of substances: (1) chemically similar compounds which share a common transport system with the test sugar, (2) metabolic poisons and (3) a diverse group of rather unrelated compounds provided they had been carbon source for growth of the cell. The first two types of inhibition are well recognized in all types of animal, plant and bacterial cells. The latter inhibition by "growth-substrate", has been described clearly only in bacteria. The explanation for this curious phenomenon is not well understood although a variety of hypotheses have been advanced.

KEPES[1] and KOCH[2] have shown that glucose inhibits the transport of β-galactosides in glucose-grown cells, but not in cells grown on other carbon sources. Metabolizable compounds have been shown to inhibit the uptake of galactose[3-5], β-galactosides[2,6] as well as the uptake of α-methylglucoside[6-11]. These observations led KEPES[1] to postulate that the metabolizable substance (glucose) and the test sugar (thiomethylgalactoside) shared a non-specific "common carrier" in the transport process (the *transporter*), which he distinguished from the specific factor, the permease. In his view, the function of the permease is to catalyze the reaction between substrate and transporter. Since there is a limited amount of this non-specific carrier in the plasma membrane, two different substrates, in the presence of their corresponding permeases, could compete with each other for entry into the cell. This has been the dominant view among workers in the field although additional hypotheses have been proposed[6,8-13].

Data are presented in this paper to support the hypothesis that the product of the "y" gene is the membrane carrier itself and not an enzyme which catalyzes the reaction between substrate and transporter. This hypothesis has been extended to explain the inhibitory effects of α-methylglucoside on β-galactoside transport.

EXPERIMENTAL PROCEDURE

Bacteria

The strain of *Escherichia coli* used in this study was ML 308 ($i^-z^+y^+$) which differs from the wild type in being constitutive for both β-galactoside transport and β-galactosidase (EC 3.2.1.23). The mineral medium used was Medium 63 (see ref. 14) *plus* NaCl which contains KH_2PO_4 (13.6 g), $(NH_4)_2SO_4$ (2.0 g), $MgSO_4 \cdot 7\ H_2O$ (0.2 g), $FeSO_4 \cdot 7\ H_2O$ (0.005 g), NaCl (2.9 g) made up with 1 l of water and adjusted to pH 7.0 with KOH. Carbon sources for growth were used at a concentration of 0.2% except for casein hydrolysate at 1%, and succinate at 0.4%.

To obtain logarithmically growing cells, 0.5 ml of an overnight cell suspension was transferred to a sidearm flask containing 30 ml of fresh medium with the indicated carbon source and grown on a rotary shaker at about 200 rev./min at 37°. These cells were then centrifuged, washed by suspending in mineral medium, again centrifuged and then suspended at a suitable density for subsequent experiments in medium containing chloramphenicol (50 μg/ml) in the absence of carbon source.

Chemicals

Thiomethyl-β-[^{14}C]galactoside was obtained from New England Nuclear Corp.;

α-methyl-D-[^{14}C]glucoside from Nuclear Chicago Corp.; D-glucose from Merck and Co.; D-fructose from Eastman Kodak Co.; 2-deoxy-D-glucose and sodium glucose 6-phosphate from the Sigma Chemical Co.; *o*-nitrophenyl-β-galactoside, thiomethyl-β-galactoside, *p*-nitrophenyl-β-glucoside and sodium gluconate from Calbiochemical Corp.; 3-*O*-methyl-D-glucose from Ayersts Laboratories; α-methyl-D-glucoside from Pfanstiehl Laboratories; arbutin (*p*-hydroxyphenyl-β-glucoside) from Aldrich Chemical Co.; thio-β-digalactoside from Mann Research Laboratories, Inc. Chloramphenicol was a gift of the Parke, Davis Co. β-Methylglucoside was a gift from Corn Products Co. 1-Deoxyglucose (1,5-anhydro-D-glucitol) was a gift from Dr. N. RICHTMYER; 6-deoxyglucose was a gift from Dr. B. R. LANDAU. α-Methylglucoside and 3-*O*-methylglucose were recrystallized from ethanol before use. All glucose analogs except 2-deoxyglucose were tested for glucose contamination using Glucostat reagent (Worthington Biochemical Corporation).

Assay of intracellular radioactivity

Washed cells were suspended at a final density of 300 μg dry wt./ml (absorbance of 150 Klett units at 420 mμ) (2.7 μl/mg dry wt.[12]) with the indicated additions. The suspension was incubated at room temperature (23°), unless otherwise indicated, and at various intervals 0.5-ml aliquots were pipetted onto the center of a Millipore filter (0.65 μ pore size) which had been pre-cooled with mineral medium at 0°. The cells were then quickly washed with 10 ml of ice-cold medium. The Millipore filter was placed directly into a liquid-scintillation vial to which was added scintillation fluid (a 12:7 mixture of toluene–ethanol, containing 0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis-(5-phenyloxazolyl-2)benzene). The sample was then counted in a liquid-scintillation counter with about 70% efficiency (Packard Tri-Carb).

Assay of α-methylglucoside and α-methylglucoside phosphate

To determine the proportions of neutral and anionic radioactivity within the cells aliquots which had been incubated with the indicated concentration of α-methyl-[^{14}C]glucoside were filtered on a Millipore filter and then extracted with boiling water (extraction with trichloroacetic acid (8%) at 0° gave similar results). The extract was cooled and applied to a 4 cm × 0.6 cm column of Dowex-1 formate. The neutral α-methylglucoside was eluted with 8–10 ml of distilled water after which no further counts could be removed with water. The sugar phosphate was then eluted with 3 ml of solution containing 0.5 M ammonium formate and 0.2 M formic acid. The eluates were counted in a scintillation counter.

Assay of o-nitrophenyl-β-galactoside transport

The *o*-nitrophenyl-β-galactoside transport activity in ML 308 was determined by measuring the rate of *o*-nitrophenol formation when intact cells were incubated in the presence of *o*-nitrophenyl-β-galactoside. This process (hydrolysis *in vivo*) has been shown to be proportional to the transport rate[15]. The rate of yellow color formation (*o*-nitrophenol) was determined by two methods. In the first method, an aliquot of the incubation mixture containing cells, *o*-nitrophenyl-β-galactoside and the indicated additions were taken at various time intervals and the reaction stopped by addition of 2 vol. of 0.7 M Na_2CO_3 at 0°. The cells were then removed by centrifuging at 4° and the absorbance at 420 mμ determined. In the second method, the

rate of hydrolysis was followed continuously in a cuvette in a Gilford spectrophotometer.

Measurements of the flux in equilibrated cells

KEPES[1] and others[12] have shown that the flux into a cell equilibrated with a β-galactoside in the external medium is the same as the initial flux of that galactoside into a cell, *i.e.*, the influx of substrate is independent of the intracellular concentration of substrate. When a tracer of radioactive substrate is added to cells which have been equilibrated with non-radioactive substrate the increase in intracellular specific activity observed can be used to measure the influx and efflux of substrate without some of the difficulties encountered in obtaining initial rates. In these experiments cells were incubated with non-radioactive 0.5 mM thiomethyl-β-galactoside with or without the addition of inhibitor as indicated. After 30 min (23°) or 40 min (10°) incubation to obtain a steady state 0.5 mM thiomethyl-β-[^{14}C]galactoside with or without the inhibitor (glucose or α-methylglucoside) was added. This addition caused a dilution of the cell density but the concentration of substrate and inhibitor in the medium as well as the steady state in the cell remained unaltered. An aliquot of this mixture was filtered on a Millipore filter at 15 sec and 30 min (23°) to determine the 15-sec and steady-state specific activity, respectively. The rate of increase in intracellular isotope with time (dT_i^*/dt) is equal to the influx of isotope $k_{in}\frac{T_o^*}{T_o}$ *minus* the efflux of isotope $k_{ef}\frac{T_i^*}{T_i}$. T_0 and T_i are the concentrations of substrate outside and inside the cells, respectively; k_{in} and k_{ef} are the influx and efflux rates which must be equal in the steady state and hence both can be expressed as k; the asterisk (*) designates the isotopic substrate. Since the ratio T_o^*/T_o is constant because of the large extracellular volume the influx of isotope is constant. All parameters except k can be measured experimentally. Integration of the following equation

$$\frac{dT_i^*}{dt} = k_{in}\frac{T_o^*}{T_o} - k_{ef}\frac{T_i^*}{T_i}$$

gives

$$T_i^* = T_i\frac{T_o^*}{T_o}\left(1 - e^{-\frac{kt}{T_i}}\right)$$

from which unidirectional flux, k ($k_{in} = k_{ef}$), can be determined.

Measurement of galactoside exit

Cells (1 ml at a density of 2 mg dry wt./ml) were incubated 30 min at room temperature with various concentrations of radioactive galactoside to obtain cells containing the high levels of intracellular galactoside. The cells were then centrifuged at 0° for 10 min, decanted, the cells resuspended in 0° medium and centrifuged again. The supernatant fluid was carefully decanted and the inside of the tube wiped free of adhering drops of radioactive medium. The pellet was then quickly taken up in 30 ml of medium at 23° with the indicated additions so that a dilute suspension of about 67 μg dry wt./ml was obtained. At various intervals 5-ml samples were removed, filtered and washed on precooled Millipore filters.

The rate of efflux could also be measured using the steady-state equation above. In this case the flux constant, k, divided by the steady-state concentration will yield the efflux per unit concentration difference.

RESULTS

General properties of the inhibition

The transport of β-galactosides by *E. coli* can be inhibited by glucose (and various glucose analogs)[1,2] even though these two transport systems are genetically distinct[15,16]. Table I shows the effect of glucose and glucose derivatives upon the

TABLE I

INHIBITION OF *o*-NITROPHENYL-β-GALACTOSIDE UPTAKE IN GLUCOSE-GROWN ML 308

o-Nitrophenyl-β-galactoside uptake was measured by continuously monitoring the hydrolysis *in vivo* of *o*-nitrophenyl-β-galactoside (1 mM) at 23° using the Gilford spectrophotometer. Results are expressed as mean values of three or more experiments. At low glucose concentrations the ratio was variable but less than 1. Utilization of glucose during the measurement presumably accounts for this variation.

Inhibitor	*Concn. (mM)*	*Rate in presence of inhibitor* / *Rate in absence of inhibitor*
α-Methylglucoside	10	0.3
	1	0.3
	0.1	0.3
	0.01	0.9
Glucose	10	0.5
	1	0.5
	0.1	approx. 0.7
1-Deoxyglucose	10	0.3
	1	0.3
	0.1	0.3
2-Deoxyglucose	10	0.3
	1	0.3
	0.1	0.3
β-Methylglucoside	10	0.3
	1	0.3
	0.1	0.3
6-Deoxyglucose	1	1.0
Glucose 6-phosphate	1	1.0
3-*O*-Methylglucose	1	1.0
Arbutin	1	1.0
p-Nitrophenol-β-glucoside	1	0.9
Fructose	1	1.0

uptake of *o*-nitrophenyl-β-galactoside into ML 308 grown on glucose as carbon source. Many, but not all, glucose analogs were inhibitory. The inhibition reached its maximum value at an inhibitor concentration between 10^{-5} and 10^{-4} M but did not approach 100% even at very high concentrations. This inhibition was not due to an inhibition of β-galactosidase since the hydrolysis of *o*-nitrophenyl-β-galactoside by a sonicated extract was not inhibited by glucose or α-methylglucoside (10 mM). Fig. 1 shows the effect of some of these compounds on the uptake of thiomethyl-β-

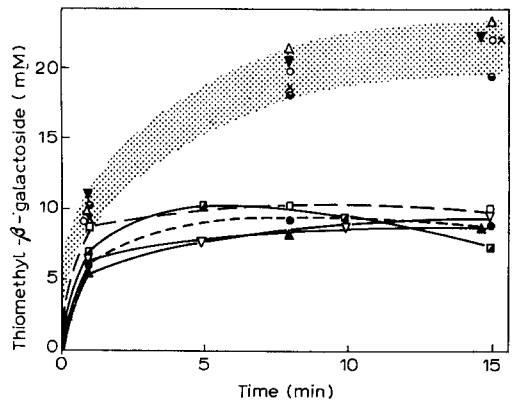


Fig. 1. Effect of glucose and glucose derivatives on thiomethyl-β-galactoside transport in glucose-grown ML 308. Washed cells were incubated in a mineral medium containing thiomethyl-β-[^{14}C]-galactoside (0.5 mM) and the uptake into the cells measured at various time intervals. The range of values from three control experiments is indicated by the shaded envelope. The inhibitors and their concentrations are as follows: 3-*O*-methylglucose at 1 mM, ×; glucose 6-phosphate at 10 mM, △; *p*-nitrophenyl-β-glucoside at 1 mM, ◒; arbutin at 1 mM, ▼; 6-deoxyglucose at 1 mM, ○; 2-deoxyglucose at 0.1 mM, □; α-methylglucoside at 1 mM, ▽; β-methylglucoside at 0.1 mM, ●; 1-deoxyglucose at 0.1 mM, ▲; and glucose at 1 mM, ◪. Each point shown represents the mean value of three experiments.

galactoside in ML 308 grown on glucose as carbon source. The same compounds, namely α-methylglucoside, β-methylglucoside, 1-deoxyglucose, 2-deoxyglucose and glucose, inhibited both thiomethyl-β-galactoside accumulation and the hydrolysis of *o*-nitrophenyl-β-galactoside *in vivo*

Specificity of the glucose transport system in ML 308

Table II shows the effect of glucose and glucose derivatives on the influx of α-methyl[^{14}C]glucoside. Those compounds which inhibit α-methylglucoside uptake

TABLE II

EFFECT OF GLUCOSE AND GLUCOSE DERIVATIVES ON UPTAKE OF α-METHYLGLUCOSIDE IN GLUCOSE-GROWN ML 308

The concentration of α-methylglucoside as well as the inhibitors was 0.25 mM. Cells were added to the substrate and inhibitor simultaneously. α-Methyl[^{14}C]glucoside uptake was measured after 1 min incubation. Values shown are taken from a representative experiment.

Inhibitor	*Per cent of control uptake*
Glucose	11
1-Deoxyglucose	27
β-Methylglucoside	27
2-Deoxyglucose	58
3-*O*-Methylglucose	100
p-Nitrophenyl-β-glucoside	93
Arbutin	98
Glucose 6-phosphate	103
6-Deoxyglucose	105
Fructose	113

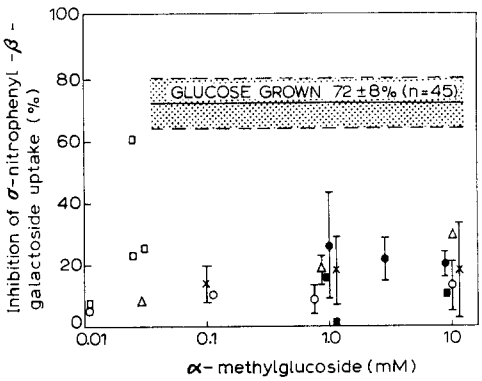


Fig. 2. Effect of α-methylglucoside on *o*-nitrophenyl-β-galactoside (1 mM) uptake in ML 308 grown on various carbon sources. Glucose 6-phosphate-grown cells (three experiments), ●; succinate-grown cells (five experiments where standard deviations are given), ○; casein hydrolysate-grown (seven experiments where standard deviation is given), △; gluconate-grown (three experiments), ×; fructose-grown, ■; glucose-grown, □, and the shaded area indicates ± 1 S.D.

were presumed to be substrates for the glucose transport system. It is evident that the compounds which appeared to be substrates for the glucose transport system were also the inhibitors of β-galactoside transport. This specificity pattern for the glucose transport system agrees with that found in *E. coli* K_{12} (refs. 7, 17) and *Salmonella typhimurium*[11].

Effect of carbon source for growth

Cells which had been grown on carbon sources other than glucose were much less sensitive to the inhibitory effects of glucose and glucose derivatives. Fig. 2 shows the effect of various concentrations of α-methylglucoside on the uptake of *o*-nitrophenyl-β-galactoside in ML 308 grown on a wide spectrum of carbon sources. Fig. 3

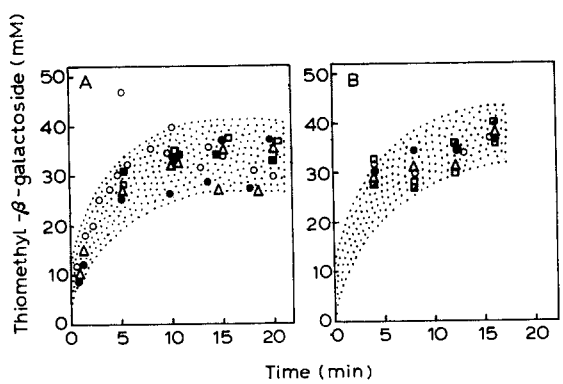


Fig. 3. The effect of growth on various carbon sources on the uptake of thiomethyl-β-galactoside in the presence of glucose and glucose derivatives. (A) ML 308 grown on casein hydrolysate; (B) ML 308 grown on succinate. Initial thiomethyl-β-[^{14}C]galactoside concentration was 0.5 mM. Compounds tested as inhibitors at a concentration of 1 mM. Control, ●; α-methylglucoside, △; glucose, ○; 3-*O*-methylglucose, □; 2-deoxyglucose, ■; and 1-deoxyglucose, ◪. Points obtained from all three experiments are shown within the shaded envelope.

demonstrates the lack of inhibition of thiomethyl-β-galactoside transport in cells grown on carbon sources other than glucose. Cells grown on casein hydrolysate or succinate (Fig. 3) as well as cells grown on gluconate were insensitive in comparison to cells grown on glucose (Fig. 1). Cells grown on glucose 6-phosphate as the source of carbon were found to have highly variable inhibitions of both *o*-nitrophenyl-β-galactoside and thiomethyl-β-galactoside transport.

The lac operon is sensitive to catabolite repression in this constitutive strain. Growth on glucose repressed the level of both the transport system and β-galactosidase to about 50% of the level of cells grown on succinate. However, growth on gluconate or growth on succinate in the presence of a poor nitrogen source, glycine, also was found to give a high level of catabolite repression but these cells were not sensitive to inhibition of β-galactoside transport by glucose and glucose derivatives. Thus, the level of catabolite repression of the lac operon did not correlate with the susceptibility of the cells to inhibition by α-methylglucoside.

Induction of the glucose transport system

The accumulation of radioactivity by cells incubated with α-methyl[^{14}C]-glucoside was greater in cells grown on glucose than in those grown on other carbon sources. Table III shows that there was a higher maximum rate of transport (v_{max}) but the same half-saturation constant (K_t) in glucose-grown cells as compared to

TABLE III

KINETICS OF α-METHYLGLUCOSIDE UPTAKE BY ML 308 GROWN ON VARIOUS CARBON SOURCES

Kinetic parameters were determined from a plot of *v versus v/S*. *v* is the initial rate of uptake as measured by uptake during the first 15 sec of incubation with α-methyl[^{14}C]glucoside. Values are expressed as mean ± S.D.; three experiments were performed with each carbon source.

Carbon source	K_t (10^{-4} *M*)	v_{max} (*μmoles per ml cell water per min*)
Glucose	2.1 ± 0.9	21.0 ± 5.0
Casein hydrolysate	2.3 ± 2.8	3.4 ± 0.5
Glucose 6-phosphate	2.5 ± 0.5	5.4 ± 0.6
Gluconate	1.7 ± 0.3	3.4 ± 1.7
Fructose	2.0 ± 0.3	5.4 ± 0.8

cells grown on other carbon sources. This is consistent with the presence of the same glucose transport system in the uninduced cells as in the induced cells but at only 20% of the fully induced level. The transport of glucose as measured with [^{14}C]-glucose 15-sec uptake showed the same pattern of induction. When the glucose transport system was induced the transport of β-galactosides was inhibited by glucose and glucose derivatives to a much greater extent than in the uninduced cells.

Kinases

The activity of the glucokinase[18] has been investigated as a function of carbon source for growth in ML 308. The activity was measured by following spectrophotometrically the reduction of TPN^+ in a reaction mixture containing 1 mM glucose,

2 mM ATP, 2 mM $MgCl_2$ and 1 μg glucose-6-phosphate dehydrogenase *plus* sonicated extract of the cells. The glucokinase was as high in fructose or casein hydrolysate-grown cells as in cells grown on glucose and hence did not correlate with the dependence of inhibition on the carbon source used for growth. α-Methylglucoside did not appear to be a substrate for glucokinase since it was not a competitive inhibitor. Furthermore, the presence of fructokinase was not a sufficient condition for inhibitability since β-galactoside transport in fructose-grown cells was not inhibited by either glucose or α-methylglucoside. However, the α-methylglucoside kinase described by KUNDIG *et al.*[19] was not assayed.

Intracellular pool of α-methylglucoside and α-methylglucoside phosphate

Since the induction of the glucose transport system enabled the cell to accumulate a higher steady-state level of α-methylglucoside and α-methylglucoside phosphate it was of interest to determine whether a large intracellular pool of the inhibitor was sufficient for the inhibition. By greatly increasing the extracellular concentration of α-methylglucoside-bathing cells grown on carbon sources other than glucose, an intracellular pool of the inhibitor could be formed whose size was of the same order

TABLE IV

THE INTRACELLULAR POOL OF α-METHYLGLUCOSIDE AND α-METHYLGLUCOSIDE PHOSPHATE

Cells were incubated for 6 min with α-methyl[^{14}C]glucoside and *o*-nitrophenyl-β-galactoside (1 mM) before filtering and extracting as described in METHODS. Values are mean ± S.D.; number of observations in parentheses. (*o*-Nitrophenyl-β-galactoside (1 mM) was present during the incubation to make conditions comparable to those when the inhibition of *o*-nitrophenyl-β-galactoside uptake was measured.)

Carbon source	*Extracellular concn. (mM) of α-methylglucoside*	*Intracellular concn. (mM)*			*Per cent of total present as α-methylglucoside*
		α-Methylglucoside	*α-Methylglucoside phosphate*	*Total*	
Glucose	0.1	3 ± 1 (5)	7 ± 1 (5)	10 ± 2 (5)	32 ± 8 (5)
Glucose	11.0	12 ± 5 (7)	8 ± 2 (7)	20 ± 4 (7)	57 ± 13 (7)
Succinate	11.0	8 ± 2 (10)	4 ± 2 (10)	12 ± 4 (10)	71 ± 13 (10)
Gluconate	11.0	10 ± 5 (10)	7 ± 1 (10)	17 ± 1 (10)	55 ± 11 (10)

as that formed by glucose-grown cells (Table IV). The fraction of the pool present as the phosphorylated derivative was also about the same as that in glucose-grown cells. The fact that even under these conditions there was no inhibition by α-methylglucoside suggests that metabolic and osmotic changes associated with the presence of these pools were not responsible for the inhibition. However, it is quite possible that the "true" inhibitor is formed only in glucose-grown cells and is not measured under these conditions. Osmotic effects seemed unlikely since neither the basal rate nor the inhibited rate of β-galactoside transport was appreciably affected by raising the osmotic strength of the medium by the addition of either KCl or sucrose.

Influx

To investigate the influx component of the accumulation, cells were added to

thiomethyl-β-[^{14}C]galactoside and after 15 sec incubation quickly filtered as described above. The influx of thiomethyl-β-galactoside into inhibited cells was studied by measuring the initial rate (15 sec) of entrance under two conditions: (1) cells were added to thiomethyl-β-[^{14}C]galactoside *plus* inhibitor, (2) cells were preincubated with the inhibitor and then these cells were added to thiomethyl-β-[^{14}C]galactoside and sampled after a further 15 sec. Fig. 4 shows the results of such an experiment. Here v versus v/S is plotted and the v_{max} and K_t can be determined. A striking

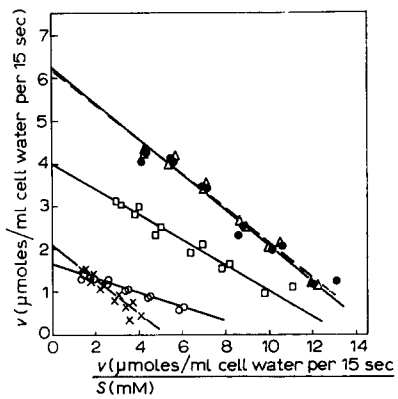


Fig. 4. Effect of α-methylglucoside and glucose on the kinetics of thiomethyl-β-galactoside influx. Cells were incubated in the presence of thiomethyl-β-[^{14}C]galactoside at various concentrations (0.1 to 1.0 mM) with or without inhibitors. Influx was measured by the uptake of radioactivity in 15 sec. Control (no inhibitor), ●; α-methylglucoside (0.1 mM) no preincubation, △— —△; glucose (0.5 mM) no preincubation, □; α-methylglucoside (0.1 mM) 12 min preincubation, ○; glucose (0.5 mM) 12 min preincubation, ×.

point is that α-methylglucoside requires a preincubation period (longer than 15 sec) to give an inhibition of influx. Glucose is inhibitory even if added simultaneously with thiomethyl-β-galactoside; the inhibition is much larger, however, after preincubation. The results are summarized in Table V.

TABLE V

EFFECT OF α-METHYLGLUCOSIDE AND GLUCOSE ON THE KINETICS OF THIOMETHYL-β-GALACTOSIDE UPTAKE IN GLUCOSE-GROWN ML 308

Kinetic constants were determined from a plot of initial 15 sec velocity, v, *versus* v/extracellular concn. Results are expressed as mean values ± S.D.; the number of experiments is given in parentheses.

Inhibitor	*Temperature*	*Preincubation (min)*	K_t (10^{-4} *M*)	v_{max} (*μmoles/ml cell water per min*)
None	23°	None	4.1 ± 0.3 (4)	24.8 ± 1.8 (4)
α-Methylglucoside (0.1 mM)	23°	None	4.2 ± 0.1 (3)	23.8 ± 1.2 (3)
α-Methylglucoside (0.1 mM)	23°	10–45	1.9 ± 0.3 (3)	5.9 ± 1.1 (3)
Glucose (0.5 mM)	23°	None	3.2 ± 0.4 (3)	14.9 ± 0.7 (3)
Glucose (0.5 mM)	23°	6	4.2 ± 0.2 (3)	8.7 ± 0.3 (3)

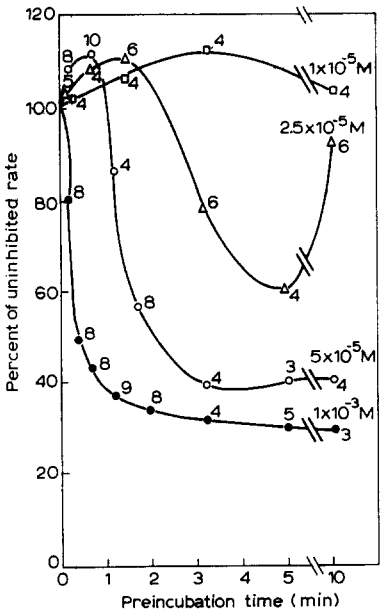


Fig. 5. Effect of preincubation time with α-methylglucoside on the influx of thiomethyl-β-galactoside. Glucose-grown cells were exposed to α-methylglucoside at the indicated concentrations for various periods before the 15-sec uptake of thiomethyl-β-[^{14}C]galactoside was measured. The number of experiments is indicated.

Fig. 5 shows the effect of the time of preincubation with the inhibitor (α-methylglucoside), on thiomethyl-β-galactoside influx. With a high concentration (1 mM) of α-methylglucoside a preincubation time of less than 1 min was sufficient to produce a marked inhibition of thiomethyl-β-galactoside transport, while with lower inhibitor concentrations a longer preincubation time was required.

A 15-sec incubation period was usually satisfactory for measuring the influx

TABLE VI

UPTAKE OF THIOMETHYL-β-GALACTOSIDE IN GLUCOSE-GROWN ML 308 AS MEASURED IN EQUILIBRATED CELLS

Thiomethyl-β-[^{14}C]galactoside influx was measured in cells previously equilibrated with non-radioactive thiomethyl-β-galactoside, by the procedure described in METHODS. The concentration of thiomethyl-β-galactoside in the medium was 0.5 mM. Values are given as mean ± S.D. The number of observations is indicated in parentheses.

Inhibitor	*Temperature*	*Influx (μmoles/ml cell water per min)*	*Steady-state concentration (μmoles/ml cell water)*
None	23°	14.4 ± 3.8 (11)	19.0 ± 2.0 (11)
α-Methylglucoside (1 mM)	23°	1.0 ± 0.3 (11)	4.0 ± 0.8 (11)
Glucose (5 mM)	23°	5.6 ± 0.8 (10)	6.7 ± 0.3 (10)
None	10°	6.6 ± 2.2 (6)	20.9 ± 0.4 (6)
α-Methylglucoside (1 mM)	10°	1.1 ± 0.1 (5)	6.1 ± 0.1 (5)
Glucose (5 mM)	10°	3.5 ± 0.6 (6)	14.5 ± 0.1 (6)

because the efflux was negligible, the intracellular pool being small in relation to the K_t of efflux. Nevertheless, the possibility was considered that the inhibitors had greatly increased the affinity of the exit reaction and that the lower intracellular pool observed after 15 sec was due to an increased efflux during this short period and not a decreased influx. However, if the cells were allowed to reach a steady state by incubating them for 30 min with non-radioactive thiomethyl-β-galactoside, with or without glucose or α-methylglucoside, and then a tracer of thiomethyl-β [^{14}C]galactoside was added without perturbing the steady state, the influx of thiomethyl-β-galactoside could be measured. In the steady state the influx must equal the efflux and the size of the intracellular steady-state pool can be measured. From these values the influx may be calculated independent of the efflux changes by the equation shown in METHODS. Data from such experiments are shown in Table VI. The results were essentially the same as those using the non-steady-state initial uptake method, *i.e.*, both α-methylglucoside and glucose were inhibitors of thiomethyl-β-galactoside influx. These data were in accord with the inhibition of *o*-nitrophenyl-β-galactoside uptake (Fig. 2) since *o*-nitrophenyl-β-galactoside uptake measures influx without a complicating efflux component.

Counterflow and reciprocity

It has been postulated that this inhibition of β-galactoside transport by

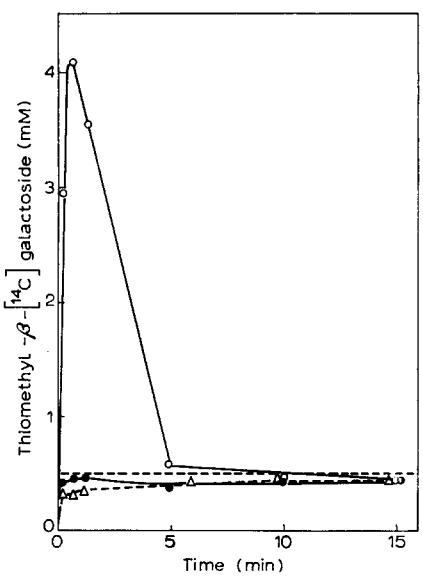


Fig. 6. Thiomethyl-β-galactoside counterflow in poisoned ML 308. Glucose-grown cells were incubated 30 min in the presence of azide (30 mM) and iodoacetate (1 mM) *plus* thiomethyl-β-galactoside (20 mM), α-methylglucoside (20 mM) or no sugar. Cells were then centrifuged 10 min at 0° to form a firm pellet. The supernatant fluid was decanted, and the tube carefully wiped. The volume of adhering fluid as measured by [^{3}H]inulin was 7–16 μl. The pellet was then resuspended in 2 ml of medium containing thiomethyl-β-[^{14}C]galactoside (0.5 mM) *plus* the two metabolic inhibitors and sampled at intervals. Control, ●; preloaded with thiomethyl-β-galactoside (20 mM), ○; and preloaded with α-methylglucoside (20 mM), △. The dashed horizontal line represents the concentration of thiomethyl-β-[^{14}C]galactoside in the medium (the equilibration level).

glucose was due to competition for transport across the membrane *via* a common carrier or transporter[1,2]. According to this view, the complexing of glucose and the β-galactosides to this carrier was catalyzed by the glucose and lactose "permeases", respectively. An experiment bearing on this question was performed with α-methylglucoside as the inhibitor. It had been shown that when a metabolically poisoned cell was preloaded with one β-galactoside, washed and added to a second galactoside, there was a large but transient accumulation against a gradient due to competition for the exit carrier between the two substrates[2,12]. If glucose-grown cells are metabolically poisoned and preloaded with α-methylglucoside, washed and exposed to thiomethyl-β-[^{14}C]galactoside, the α-methylglucoside should cause a transient accumulation if it has affinity for the same carrier as thiomethyl-β-galactoside. A typical result of such a series of experiments is shown in Fig. 6. Similar results were obtained with either azide alone or azide *plus* iodoacetate. Preloading with α-methylglucoside did not cause the "counterflow" of thiomethyl-β-galactoside seen with β-galactoside-preloaded cells. The presence of a high concentration of α-methylglucoside in the preloaded cells was established by performing parallel experiments with α-methyl[^{14}C]glucoside.

Further evidence that the glucosides and β-galactosides are not substrates for

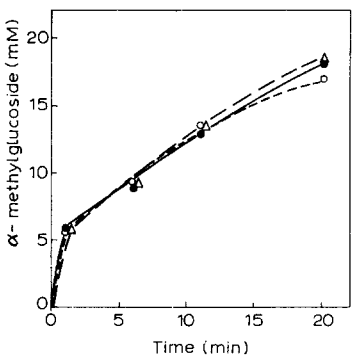


Fig. 7. Effect of β-galactosides on the uptake of α-methylglucoside in glucose-grown ML 308. α-Methyl[^{14}C]glucoside concentration was 0.25 mM. β-Galactosides were added at a concentration of 1 mM. Cells were added to substrate and inhibitor simultaneously. Control, ●; thiomethyl-β-galactoside, ○; thio-β-digalactoside, △.

the same system is shown in Fig. 7. In this experiment the transport of α-methyl-[^{14}C]glucoside was measured in glucose-grown cells in the presence and absence of galactosides. The thiogalactosides, thiomethyl-β-galactoside and thio-β-digalactoside, did not inhibit the transport of α-methylglucoside. Thio-β-digalactoside has the highest affinity for the β-galactoside transport system known. The lack of reciprocity of inhibition, such as demonstrated here, puts severe restrictions on the hypothetical common element in these transport systems (see DISCUSSION).

Efflux

Knowledge of the effect of the inhibitors on the exit of β-galactosides from the cells was important for the elucidation of the mechanism of inhibition. Two types

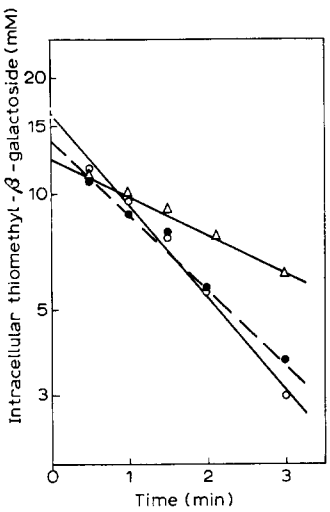


Fig. 8. Exit of thiomethyl-β-galactoside from glucose-grown ML 308. Cells were preloaded with thiomethyl-β-[^{14}C]galactoside for 30 min at 23°, centrifuged, washed with sugar-free medium at 0°, centrifuged and resuspended in the indicated solution at 23°. Control, ●; α-methylglucoside (1 mM), △; glucose (5 mM), ○. Summary of values in Table VII.

of efflux measurements have been made. The first was a direct measure of the half-time ($t_{\frac{1}{2}}$) of exit from cells preloaded with thiomethyl-β-[^{14}C]galactoside. After preloading the cells were centrifuged, washed and resuspended in media without substrate but containing α-methylglucoside or glucose. The loss of thiomethyl-β-galactoside from the cells was measured. The second method was to calculate the exit rate in cells in the steady state as was done for influx (see METHODS). In the steady state the efflux and influx are equal and the exit rate constant (μmoles/ml cell water per

TABLE VII

EFFLUX OF THIOMETHYL-β-GALACTOSIDE FROM GLUCOSE-GROWN ML 308

Control exit rate in the direct measurement is taken as 1.00, the average half-time of exit being 1.7 ± 0.6 min. Results are expressed as mean values ± S.D.; number of observations are in parentheses.

Inhibitor	*Efflux from steady-state measurements* (μmole/ml cell water per min per mM concentration difference)*	*Relative efflux from direct measurement** (relative half-time)$^{-1}$*
None	0.76 ± 0.25 (11)	1.00 (6)
Glucose	0.76 ± 0.25 (10)	1.30 ± 0.02 (5)
α-Methylglucoside	0.38 ± 0.01 (11)	0.55 ± 0.02 (6)

* Value determined using equation in EXPERIMENTAL PROCEDURE and data in Table VI (see text).

** Values are taken from experiments such as shown in Fig. 8.

min per mM concentration difference) is equal to the influx divided by the steady-state intracellular concentration. A typical experiment demonstrating the first method, the direct measurement of exit, is shown in Fig. 8. The results with both methods are summarized in Table VII. Both methods indicated that not only the influx but also the efflux was inhibited by α-methylglucoside.

Inhibition in energy-uncoupled cells

To test the possibility that the site of inhibition of α-methylglucoside on galactoside transport was at the level of energy coupling, various experiments were performed with metabolic inhibitors. First the effect of metabolic inhibitors on transport of α-methylglucoside and thiomethyl-β-galactoside was examined. The effect of azide (30 mM), 2,4-dinitrophenol (1 mM) and azide (30 mM) *plus* iodoacetate (1 mM) on the accumulation of α-methyl[^{14}C]glucoside is shown in Fig. 9. The addition of iodoacetate blocked the transport of α-methylglucoside; azide or 2,4-dinitrophenol alone did not appreciably inhibit the accumulation of α-methylgluco-

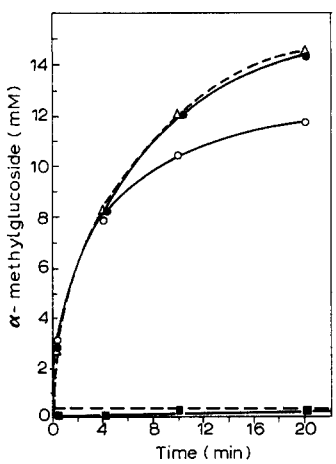


Fig. 9. The effect of metabolic inhibitors on the accumulation of α-methyl[^{14}C]glucoside in glucose-grown ML 308. Cells were incubated with the indicated inhibitors 30 min before the addition of substrate. The dashed line represents the medium concentration of α-methyl[^{14}C]-glucoside. Azide (30 mM) *plus* iodoacetate (1 mM), ■; azide (30 mM), ○; 2,4-dinitrophenol (1 mM), △; and control, ●.

side. In contrast, all were potent inhibitors of β-galactoside active transport[12].

If the action of α-methylglucoside were to uncouple energy supplies from thiomethyl-β-galactoside transport, then cells already uncoupled should be quite insensitive to α-methylglucoside. The effects of glucose and α-methylglucoside on various β-galactoside fluxes in azide-poisoned cells are shown in Table VIII. *o*-Nitrophenyl-β-galactoside influx was markedly inhibited by both α-methylglucoside and glucose in azide-poisoned glucose-grown cells while there was no effect in similarly treated casein hydrolysate-grown cells. The 15-sec thiomethyl-β-galactoside uptake in energy-uncoupled cells is quite adequate for comparative purposes as a measure of influx even with the appreciable efflux component found under these conditions[12]. α-Methylglucoside showed a 46% inhibition of thiomethyl-β-galactoside

TABLE VIII

EFFECT OF α-METHYLGLUCOSIDE AND GLUCOSE ON β-GALACTOSIDE FLUXES IN AZIDE (30 mM) POISONED CELLS

Results are expressed as mean values ± S.D.; the number of experiments is given in parentheses.

Substrate (1 mM)	*Flux*	*Carbon source for growth*	*Inhibitor (1 mM)*	*Per cent of control*
o-Nitrophenyl-β-galactoside	Influx (*in vivo* hydrolysis)	Glucose	α-Methylglucoside	33 ± 6 (7)
			Glucose	28 ± 12 (7)
o-Nitrophenyl-β-galactoside	Influx (*in vivo* hydrolysis)	Casein AA	α-Methylglucoside	100 (2)
			Glucose	100 (2)
Thiomethyl-β-galactoside	Influx (15 sec uptake)	Glucose	α-Methylglucoside	54 ± 14 (26)
			Glucose	82 ± 15 (21)
Thiomethyl-β-galactoside	Influx (15 sec uptake)	Casein AA	α-Methylglucoside	101 ± 7 (8)
			Glucose	161 ± 17 (8)
Thiomethyl-β-galactoside	Efflux $(\text{half-time})^{-1}$	Glucose	α-Methylglucoside	18 ± 6 (4)
			Glucose	30 ± 9 (4)
Thiomethyl-β-galactoside	Efflux $(\text{half-time})^{-1}$	Gluconate	α-Methylglucoside	85 (3)
			Glucose	98 (2)

uptake in azide-poisoned, glucose-grown cells. Glucose, on the other hand, showed only an insignificant inhibition of thiomethyl-β-galactoside uptake under these conditions. Any interpretation of this effect of glucose must take into account the fact that glucose can supply energy *via* glycolytic pathways in the presence of azide and hence lead to active transport. This glycolytic stimulation was very apparent in the casein hydrolysate-grown cells where the addition of glucose caused a 61% increase in thiomethyl-β-galactoside uptake while the non-metabolizable α-methylglucoside had no effect. Thus, the absence of an observed stimulation or inhibition by glucose in glucose-grown cells may be due to a balance of α-methylglucoside-like inhibition and glycolytic stimulation. Thus, these results have shown that the mechanism of inhibition of β-galactoside transport is independent of the energy coupling.

DISCUSSION

The inhibitory effect of a growth-substrate on the transport of galactosides[2,6], α-methylglucoside[6–11], and galactose[3–5] is well documented. In the present investigation an attempt has been made to test various hypotheses designed to explain this phenomenon. The inhibitor chosen for most of the work was α-methylglucoside which apparently undergoes only two reactions, active transport into the cell and phosphorylation to give the α-methylglucoside 6-phosphate[17,7]. It was hoped that the very limited metabolism of α-methylglucoside would make interpretation of results somewhat simpler than with glucose or other metabolizable growth-substrates. A few of the many possible hypotheses will be considered below.

Energy-requiring exit system hypothesis

HOFFEE, ENGLESBERG and co-workers[8–11] have shown that addition of the growth-substrate reduced the steady-state level of transported α-methylglucoside in

E. coli and *S. typhimurium*. This inhibitory effect of added growth-substrate was abolished by the simultaneous addition of certain concentrations of azide or dinitrophenol. They also found that at these concentrations of metabolic inhibitors the metabolism of growth-substrate was strongly inhibited, while α-methylglucoside transport (without added substrate) was largely unaffected. On the basis of these observations HOFFEE, ENGLESBERG and co-workers postulated that the addition of growth-substrate provided ATP for a specific energy-requiring exit transport system. Although no experimental data have been presented to refute this hypothesis for α-methylglucoside transport, considerable data make this view unlikely for β-galactoside transport. KOCH[2], for example, showed that both entry and exit processes were induced simultaneously and exactly in parallel. WINKLER AND WILSON[12] have presented additional support for the belief that entry and exit of β-galactoside involve the same specialized transport apparatus.

KEPES[13] has offered an alternative explanation for the data of HOFFEE, ENGLESBERG and co-workers. He proposed that the rapid metabolism of added growth-substrate increased the level of ATP which acted directly on the intracellular portion of the transport system to inhibit accumulation. It has been suggested[12] that a "feedback" inhibitor might act to uncouple the energy from transport leading to an increased rate of exit from the cell.

While feedback inhibition provides a satisfactory explanation for many cases of growth-substrate inhibition of transport, another explanation must be sought for the inhibition of β-galactoside transport by α-methylglucoside in glucose-grown cells. Addition of α-methylglucoside to cells cannot lead to increased ATP levels. On the contrary, energy would be required for its transport and for its phosphorylation. Furthermore, α-methylglucoside inhibits β-galactoside transport whether or not metabolism is inhibited. Table VIII shows that the presence of 30 mM azide has little effect on the inhibition of *o*-nitrophenylgalactoside or thiomethyl-β-galactoside transport by α-methylglucoside. In the presence of this metabolic inhibitor ATP levels must be reduced as no active transport of any β-galactoside is possible[12]. The transport and phosphorylation of α-methylglucoside could only reduce still further the meager stores of ATP found in these cells. Thus, energy coupling or ATP levels are probably not involved in α-methylglucoside inhibition of galactoside transport.

Catabolite repression hypothesis

Some factor related to the growth of cells on glucose results in susceptibility of β-galactoside transport to inhibition by α-methylglucoside. Growth of ML 308 on glucose results in a 4–5-fold increase in α-methylglucoside transport compared with casein-grown cells. The high level of α-methylglucoside or α-methylglucoside phosphate within the cells, however, does not appear to be the sole factor in the inhibition as similar levels may be obtained under conditions in which no inhibition was observed (Table IV).

Catabolite repression is apparently not a sufficient condition for the inhibitory effect of α-methylglucoside on β-galactoside transport as conditions of high catabolite repression (without glucose) do not give cells which are susceptible to α-methylglucoside. Finally there is no obvious correlation with kinase levels. Fructose-grown cells were found to possess high levels of glucokinase and must also have high levels of fructokinase (hexokinase)[18] but did not show the inhibition of β-galactoside trans-

port by α-methylglucoside nor the induction of the glucose transport system found in glucose-grown cells.

These data are consistent with some relationship between the inhibition of β-galactoside transport by α-methylglucoside and the activity of the glucose transport system.

Osmotic swelling hypothesis

Addition of metabolizable or transportable substrates to bacteria usually leads to an increased intracellular osmotic pressure due to accumulation of the substrate itself or metabolic products. It is conceivable that swelling proceeds to the stage of stretching the plasma membrane which increases the pore size with the consequent leakage of accumulated solutes[24]. Although swelling of the cell does occur in the presence of many different metabolizable substances factors tending to prevent this swelling did not prevent the inhibition of transport by metabolizable substances. All attempts to prevent the inhibitory effect of α-methylglucoside and other substances on ML 308 by adding salts or sucrose to the external medium have failed. Furthermore, large intracellular pools of α-methylglucoside and α-methylglucoside phosphate could be induced under certain conditions without any inhibition of β-galactoside transport (Table IV).

Transporter (or common carrier) hypothesis

KEPES[1] first proposed that a specific permease catalyzed the reaction between substrate and a non-specific transporter in the plasma membrane. According to this view a variety of different carbohydrates, each with their own permease, competed with each other for the limited amount of non-specific transporter. One restriction of this hypothesis of KEPES must immediately be made on the basis of the studies of HERZENBERG[14] and the present work, namely, that the free sugars do not have a measurable affinity for the transporter ("common carrier"). HERZENBERG[14], for example, has shown that thiodigalactoside, a potent inhibitor of β-galactoside transport, has no effect on *o*-nitrophenyl-β-galactoside uptake by ML 35, a permease-negative organism. This organism would be expected to possess "common carrier" although it lacked the specific β-galactoside transport system. ML 35 also does not show another feature of membrane carriers, the "counter-flow" phenomenon which is seen in transport-positive organisms[12].

A different view of the interaction is that the substrate has affinity for the transporter only in the presence of its specific permease. This model[1,2] visualizes two substrates with separate permeases utilizing the same transporter. A mutual inhibition between the two substrates α-methylglucoside and thiomethyl-β-galactoside would be predicted. This reciprocity was not found. Neither thiomethyl-β-galactoside nor thio-β-digalactoside had an effect on α-methylglucoside influx in either glucose- or casein hydrolysate-grown cells indicating that if they share a common carrier their affinities must be markedly different. The measured affinities of α-methylglucoside and thiomethyl-β-galactoside uptake by ML 308 are comparable and that of thio-β-digalactoside much greater than α-methylglucoside but these affinities may not measure the affinity for the common element in transport. Finally, the above hypothesis would predict that counterflow would be observed with the substrate pair of α-methylglucoside and thiomethyl-β-galactoside. Although various

β-galactoside pairs show this phenomenon very clearly[12], preloading of cells with α-methylglucoside does not result in counterflow of thiomethyl-β-galactoside (Fig. 6).

Although it is clear from the previous discussion that the hypothetical "common carrier" cannot have appreciable affinity for the free sugars, some type of common element in transport seems likely. The α-methylglucoside inhibition of *o*-nitrophenyl-β-galactoside transport in either control or poisoned cells (glucose-grown) would be consistent with some common element in the transport of the two different sugars. Energy-coupling factors are eliminated in these experiments as are factors related to the extensive metabolism of the inhibitor. The requirement for preincubation of cells with α-methylglucoside prior to addition of galactoside suggests some intracellular event such as accumulation of α-methylglucoside, α-methylglucoside phosphate or an unidentified intermediate which requires growth on glucose to be formed. How this accumulation might affect a common carrier is, as yet, not fully understood. That some common element is present is suggested by recent studies of EGAN AND MORSE[20-22] which indicated that in *Staphylococcus aureus* there is a genetically distinct factor which is common to the transport of many carbohydrates. Mutants which lack this common factor cannot grow on and cannot transport lactose, maltose, sucrose, fructose, galactose, mannitol, and ribose; growth of the mutant on glucose is normal. Since each of these sugars has a separate and distinct transport system (permease), EGAN AND MORSE conclude that this new factor must be the previously postulated "common carrier".

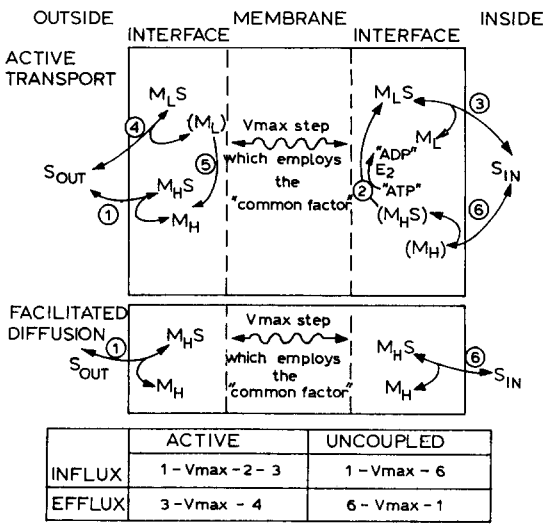
"Common factor" model

The data presented require a common element in the transport of α-methylglucoside and the β-galactosides. The lack of reciprocity and counterflow observed between these two systems suggests that the affinity for the common element possessed by α-methylglucoside is much greater than that of the β-galactosides and is not that affinity measured by the overall transport processes which are roughly equivalent for α-methylglucoside and thiomethyl-β-galactoside. The transporter or common carrier hypothesis as described above can adequately explain this data as long as the product of the "y" gene is not the carrier. If one believes that the product of the "y" gene is the galactoside carrier (as assumed for the *M*-protein of FOX AND KENNEDY[25]) then a common element which does not employ a common carrier is necessary to explain the data.

A "common factor" required by the carrier–substrate complex and common to several transport systems would meet these requirements. The "factor" has absolutely no interaction with the substrate alone. This hypothesis envisages an interaction between the substrate–carrier complex and a "factor" which is limited in amount and common to several different transport systems. Unoccupied carriers do not require a "factor" for mobility and do not have affinity for the "factor". When cells are glucose-grown, maximum amounts of glucose carrier are induced. In the presence of saturating α-methylglucoside concentrations the substrate–carrier complex combines with a major portion of the "factor" in the membrane, the α-methylglucoside–carrier complex having a much greater affinity for the "factor" than complexes of the β-galactoside transport systems (these affinities are not those measured when the overall affinity of the transport system is determined). Thus, when a major fraction of the "factor" is associated with the α-methylglucoside–

carrier complex other transport systems are unable to function at their maximal capacity due to limiting amounts of this "factor". One attractive aspect of the hypothesis is that it recognizes the product of the "y" gene as a membrane carrier itself and not as an enzyme which catalyzes the reaction between substrate and carrier (transporter).

Fig. 10 is the simplest model for the transport of β-galactosides which, in our opinion, is consistent with all the known properties of the transport system. The upper diagram shows a complete active transport system; the lower diagram, an energy-uncoupled facilitated diffusion system. M, the designation of FOX AND KENNEDY[25] for the carrier is used in this figure. The substrate is designated S. Previous work from this laboratory[12] has shown that the energy coupling enables the cell to form a concentration gradient by increasing the K_t of exit, *i.e.*, presumably a conversion of the M protein from a high (M_H) to a low (M_L) affinity carrier. At the outer surface of the cell the conversion of low-affinity carrier to a high-affinity



	ACTIVE	UNCOUPLED
INFLUX	1 - Vmax - 2 - 3	1 - Vmax - 6
EFFLUX	3 - Vmax - 4	6 - Vmax - 1

Fig. 10. Model for active transport and "facilitated diffusion" of β-galactosides. The upper portion of the figure represents active transport, the lower portion represents energy-uncoupled "facilitated diffusion". S represents the substrate; M is the carrier (product of the "y" gene), the subscripts H and L indicate the high- and lower-affinity forms of the carrier; MS is a carrier–substrate complex; E_2 is the energy-coupling enzyme which converts the high-affinity M to the lower-affinity form at the inner surface of the membrane. The compounds in parentheses are present at very low concentrations due to the actions of E_2 and its reversal[5]. The tabulation at the bottom of the figure shows those steps involved in influx and efflux in active transport and facilitated diffusion. It is hypothesized that the v_{max} step in the transport of β-galactosides involves a cofactor which is also used for the transport of α-methylglucoside and glucose. It is assumed that the v_{max} for each form of the carrier is the same.

carrier may be either enzymic[25] or spontaneous. Reactions 2 and 5 are assumed to be very rapid so that in their presence the concentrations of M_L at the outer boundary and M_H and M_HS at the inner boundary are extremely low. In the active transport system Reactions 1, 2, 3 and the v_{max} step are primarily involved in influx, and

Reactions 3, 4 and v_{max} in efflux. In the facilitated diffusion system Reactions 1, 6 and the v_{max} step are responsible for both influx and efflux. Reactions 1, 6 and the v_{max} step are identical in both active transport and facilitated diffusion systems[12]. In this model the rate-limiting step, that which determines v_{max}, is either the diffusion of the mobile carrier complexes across the membrane, or a configurational change in the carrier[26]. The K_t of entry and exit are determined by relatively rapid Reactions 1 and 3 in the active transport system and 1 and 6 in the facilitated diffusion system, respectively. The K_t of 1 and the K_t of 6 have been shown to be the same in the energy-uncoupled cell[12].

The evidence presented in this paper on α-methylglucoside inhibition of β-galactoside transport demonstrates that both influx and efflux are inhibited in both active and uncoupled transport indicating a reduction in the v_{max} step of the carrier or the reduction in the number of effective carriers. α-Methylglucoside does not apparently interact with the carrier directly but in some indirect manner *via* some component common to both transport systems. The chemical nature of this common component remains to be elucidated.

Two separate mechanisms of transport inhibition

There appear to be at least two modes of inhibition of the transport systems due to added growth-substrates (or their analogs); first, an inhibition directly related to the metabolism of the added growth-substrate. In the case of glycerol inhibition of α-methylglucoside transport in glycerol-grown cells, for example, a common carrier in transport is excluded as glycerol appears to enter the cell by simple passive diffusion[23]. KEPES[13] has suggested the hypothesis that in such cases ATP produced by metabolism inhibits the transport system at some allosteric site to produce a type of feedback inhibition. In our view this is an attractive working hypothesis. The second type of inhibition is illustrated by the α-methylglucoside inhibition of galactoside transport in glucose-grown cells. Under these conditions ATP levels in the cell are depleted and extensive metabolism does not occur. Perhaps some "common factor" in transport is affected. We suspect that glucose is a particularly effective inhibitor because it exerts its effect through both of these two different pathways. These two types of control mechanisms, although imperfectly understood at present, are probably important to the physiological regulation of transport activity and consequently to the flow of substrates into the cell.

ACKNOWLEDGEMENT

This study was carried out with the technical assistance of CAROLYN MARGOLUS.

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