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Effect of Lithium Ion on Melibiose Transport in *Escherichia Coli*

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Summary. Both Li^+ and Na^+ stimulated the uptake of thiomethylgalactoside by the melibiose transport system of *Escherichia coli.* On the other hand, Li^+ inhibited the growth of cells on melibiose as a sole source of carbon. This inhibition was specific for melibiose, and Li^+ had no effect on growth of cells on glucose, galactose, lactose, or glycerol. The effect of the cation on melibiose transport was investigated in a mutant which cannot utilize glucose. After entry into this cell, melibiose is cleaved into glucose and galactose by α-galactosidase, and the resulting glucose is excreted. Since the entry step was found to be rate-limiting, glucose production could be taken as a measure of melibiose transport. Li^+ inhibited the transport of melibiose, but not the induction of the melibiose operon nor the activity of α-galactosidase. Li^+ was found to inhibit the entry of *p*-nitrophenyl-α-D-galactoside, but not *p*-nitrophenyl-β-D-galactoside entry. Thus, the cation specificity for the melibiose membrane carrier varies with different transport substrates.

An inducible transport system for melibiose in *Escherichia coli* was discovered by Prestidge and Pardee (1965) who showed that thiomethylgalactoside (TMG) was accumulated by melibiose grown cells which did not possess a lactose transport system. They found that in many K_{12} strains the melibiose transport system was temperature-sensitive (active at 30 °C but not at 37–42 °C). In spite of this defect, such cells retain their capacity to grow on melibiose at 37 °C since this disaccharide enters the cell via the lactose transport system, induces both lactose and melibiose operons, is split by α-galactosidase and the hexoses metabolized (Beckwith, 1963). The energy coupling to melibiose transport in *Salmonella typhimurium* was investigated several years later by Stock and Roseman (1971) who demonstrated that TMG transport via the melibiose carrier was stimulated by Na^+ and Li^+. Furthermore, they found that the uptake of Na^+ by the cell was stimulated by the addition of TMG. These studies led them to suggest that melibiose was transported via cotransport with Na^+. These studies have recently been extended by Tokuda and Kaback (1977) who showed that a membrane potential

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(inside negative) induced by a potassium diffusion potential could provide the driving force for Na^+-TMG cotransport by membrane vesicles of *S. typhimurium.* They showed that the pH gradient (outside acid) produced by the respiratory proton pump could drive Na^+ extrusion by a Na^+-H^+ exchange system. Thus, in respiring cells both electrical and chemical driving forces provide the energy for the inward movement of Na^+. A similar cation-TMG cotransport system has been demonstrated in *E. coli* (Tsuchiya, Raven & Wilson, 1977; Lopilato, Tsuchiya & Wilson, 1978). It was shown that an inwardly directed electrochemical potential difference for Li^+ or for Na^+ could drive TMG accumulation by the melibiose system of energy-depleted cells of *E. coli.*

This paper describes the unexpected finding that Li^+ is a potent inhibitor of melibiose transport in *E. coli.* Furthermore, the effects of Li^+ and Na^+ vary with several different substrates for this transport system.

Materials and Methods

Bacterial Strains and Growth Conditions

Bacterial strains used in this investigation are listed in Table 1. It should be emphasized that the K-10 strains 7 and 7–6 possess a melibiose transport system stable at 37 °C, while the K-12 strains possess temperature-sensitive transport. To obtain melibiose carrier

Table 1. Summary of strains

Bacterial strains	Pertinent character[a]	Reference
E. coli K-10		
7		Hayashi *et al.*, 1964
7-6	*lacY*$^-$	Tsuchiya *et al.*, 1977
E. coli K-12		
3300	*melB*^{ts}, *lacI*$^-$	[b]
GN2	*melB*^{ts}, *lacI*$^-$, *ptsI*$^-$, *glk*$^-$	Fraenkel *et al.*, 1964
GN22	*melB*^{ts}, *lacI*$^-$ *Y*$^-$, *ptsI*$^-$, *glk*$^-$	This paper
GN22HA	*lacI*$^-$ Y$^-$, *ptsI*$^-$, *glk*$^-$	This paper
GN22LI	*melB*^{ts} (Li^+ resistant), *lacI*$^-$ *Y*$^-$, *ptsI*$^-$, *glk*$^-$	This paper

[a] Abbreviations used:
melB^{ts}: melibiose transport system is temperature sensitive (active at 30 °C and inactive at 37 °C).
lacY$^-$: lactose transport negative.
lacI$^-$: lactose repressor negative (constitutive).
ptsI$^-$: lacks enzyme I of phosphotransferase system
glk$^-$: glucokinase negative

[b] Isolated in the laboratory of J. Monod.

activity in these K-12 strains, cells were grown at 30 °C, and transport activity was measured at room temperature.

The cells were grown in Medium 63 of Cohen and Rickenberg (1956) which contains KH_2PO_4 (13.6 g), $(NH_4)_2SO_4$ (2.0 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), $FeSO_4 \cdot 7H_2O$ (0.005 g), and H_2O (1 liter) adjusted to pH 7.0 with KOH. For most experiments cells were grown in Medium 63 supplemented with 0.2% casamino acids (Difco), 10 mM melibiose and 0.5 µg/ml thiamine. Growth was followed with a Klett-Summerson colorimeter with a purple filter (No. 42).

Mutant Isolation

Strain GN22 is a lactose transport negative (*lacY*$^-$) derivative of strain GN2 Fraenkel, Falcoz-Kelly & Horecker, 1964) obtained with the method of Müller-Hill, Crapo, and Gilbert (1968) utilizing *o*-nitrophenyl-β-thiogalactopyranoside. GN2 cannot utilize glucose as it is both glucokinase negative and enzyme I negative. This cell possesses a temperature-sensitive melibiose transport system of the type found in many other K-12 strains of *E. coli* (Prestidge & Pardee, 1965). The *lacY*$^-$ derivative GN22 will grow on melibiose at 30 °C but not at 37 °C due to the temperature sensitivity of the melibiose transport protein. GN22HA and GN22LI were isolated from GN22. A culture of GN22 was mutagenized with N-methyl-N′-nitro-N-nitrosoguanidine by the method of Adelberg, Mandel & Chen (1965). GN22HA was isolated as a colony which grew on a melibiose-minimal plate at 37° and was found to possess a temperature-stable melibiose transport system. GN22LI was isolated from mutagenized GN22 by growth on melibiose-minimal plate containing 10 mM LiCl at 30°. GN22HA and GN22LI were both checked for lactose transport and found to be negative.

Transport of TMG, Raffinose, and Alanine

Washed cells were resuspended at a concentration of approximately 0.3 mg dry wt of cells per ml in 0.1 M morpholinopropane sulfonic acid (MOPS) (pH adjusted to 7.0 with tetramethyl ammonium hydroxide). The reaction was initiated by the addition of labeled substrate to a final concentration of 50 µM in the cases of TMG and raffinose, and 10 µM for L-alanine. Aliquots (0.2 ml) were removed at 30 sec, filtered, and washed with 5 ml of buffer. The filter was counted in a liquid scintillation counter.

Measurement of Melibiose Entry

Melibiose uptake was measured in cells of strain GN2 (or one of its derivatives) which cannot utilize glucose. The disaccharide enters the cell via its membrane carrier, is split to glucose and galactose by α-galactosidase, and the glucose is quantitatively excreted into the incubation medium. This method is based on a similar procedure developed by Maloney and Wilson for lactose uptake (*unpublished observations*). Cells were washed twice with Medium 63 containing 100 µg/ml of chloramphenicol, resuspended in the same medium, and incubated in the water bath shaker at 30°. The reaction was initiated by the addition of melibiose to give a final concentration of 10 mM. After various incubation periods, samples (0.5 ml) were removed and added to tubes containing 2.0 ml of ice-cold water and 0.5 ml of 0.3 N $Ba(OH)_2$. One half ml of 5% $ZnSO_4 \cdot H_2O$ was then added, and the precipitate removed by centrifugation. The concentration of free glucose in the supernatant was determined using the glucose oxidase reagent of Worthington.

Entry of α-or β-PNPG

Induced cells of GN22 were washed twice with 0.1 M potassium phosphate (pH 7.0) and resuspended in the same buffer containing chloramphenicol (100 μg/ml) plus dithiothreitol (1 mM). After preincubation at 30° for 10 min, α- or β-PNPG was added to give a final concentration of 0.5 mM and the reaction vessels shaken at 30°. Samples (3 ml) of the incubation mixture were withdrawn and placed in a tube containing 3 ml of 0.6 M Na_2CO_3. After filtration the yellow color of the *p*-nitrophenol was monitored with Klett-Summerson colorimeter (No. 42 filter).

α-Galactosidase Assay

α-Galactosidase was assayed by the procedure of Schmitt and Rotman (1966) and Burstein and Kepes (1971) using *p*-nitrophenyl-α-D-galactopyranoside (α-PNPG) as a substrate. Cells were washed with 50 mM tris (hydroxymethyl) aminomethane (Tris)-HCl at pH 7.6, and suspended in 1 ml of assay mixture containing 50 mM Tris-HCl at pH 7.5, 5 mM $MnSO_4$, 50 mM 2-mercaptoethanol, and 10 mM α-PNPG. After incubating at 37° for various time intervals, the reaction was stopped by the addition of 4.5 ml of 0.6 M Na_2CO_3 and 0.2 ml of 0.1 M ethylenediamine tetraacetic acid. The amount of *p*-nitrophenol liberated was determined with the Klett-Summerson colorimeter (filter No. 42). Under the conditions of this assay (high substrate concentration) the enzyme and not transport is rate limiting. The majority of the α-PNPG enters the cell by pathways other than that mediated by the membrane carrier.

Determination of Melibiose

For the determination of melibiose in the presence of glucose and galactose, the free reducing sugars were reduced with borohydride, as suggested by Asensio, Avigad and Horecker (1963) and the remaining α-D-galactopyranosyl-glucitol was determined with the anthrone reaction (Roe, 1954).

Chemicals

Melibiose, *p*-nitrophenyl-α-D-galactopyranoside (α-PNPG), *p*-nitrophenyl-β-D-galactopyranoside (β-PNPG) and morpholinopropane sulfonic acid (MOPS) were purchased from Sigma Chemical Co. Glucostat was from Worthington Biochemical Co. [^{14}C]-TMG, [^{14}C]-L-alanine and [^{3}H]-raffinose were purchased from New England Nuclear Co.

Results

Previous experiments with the melibiose system indicated that Li^+ (as well as Na^+) stimulated transport of the nonmetabolizable melibiose analog thiomethylgalactoside (Stock & Roseman, 1971; Tokuda & Kaback, 1977; Tsuchiya *et al.*, 1977; Lopilato *et al.*, 1978). It therefore came as a surprise that the presence of 10 mM lithium prevented growth of strain 7-6 on melibiose (Table 2). Furthermore, NaCl (10 mM) caused a slight inhibition of growth on melibiose. This strain possesses a tempera-

Table 2. Effect of Li^+ and Na^+ on growth of cells on melibiose[a]

Strain	Carbon-source	Doubling time (hr)		
		control	+10 mM NaCl	+10 mM LiCl
7-6	Glucose	1.2	1.2	1.2
	Galactose	2.3	2.3	2.9
	Melibiose	1.5	2.1	No growth
	Glycerol	1.5	1.5	1.5
7	Melibiose	1.5	1.6	2.0
	Lactose	1.3	1.3	1.5

[a] Cells were grown in Medium 63, 0.2% carbon source and 0.5 μg/ml thiamine at 37°. Where indicated NaCl (10 mM) or LiCl (10 mM) was added to growth medium.

ture-stable melibiose transport system and therefore grows well on this disaccharide at 37 °C. Li^+ was not exerting a nonspecific toxic effect on growth *per se* since this ion had no effect on the growth of these cells on glucose or glycerol and only a very slight effect on the growth on galactose. The growth of GN22 on melibiose at 30 °C was also blocked by Li^+ (data not shown).

These results indicate that Li^+ does not affect the primary metabolic pathways of the cell and suggest three possible mechanisms for the cation inhibition: (i) Li^+ inhibits induction of the melibiose operon; (ii) Li^+ inhibits the activity of α-galactosidase; (iii) Li^+ inhibits the transport of melibiose. The first two possibilities were ruled out since Li^+ had no effect on the induction or activity of α-galactosidase under several different experimental conditions. In the first experiment Li^+ had no effect on the growth on melibiose of strain 7 which possesses, in addition to the melibiose carrier, a lactose carrier (*lacY*$^+$) which transports melibiose and is insensitive to Li^+. Melibiose enters this cell via the lactose transport system, induces α-galactosidase, and is metabolized. In the second experiment Li^+ failed to inhibit the growth of strain 3300 on melibiose at 37°. In this strain the melibiose carrier is inactive at this temperature, and melibiose enters the cell via the constitutive lactose carrier. The disaccharide accumulates within the cell and induces α-galactosidase. A direct assay of α-galactosidase in strains 7 and GN22 showed that Li^+ did not affect the activity of the enzyme. These experiments are consistent with the view that Li^+ blocks the melibiose transport system.

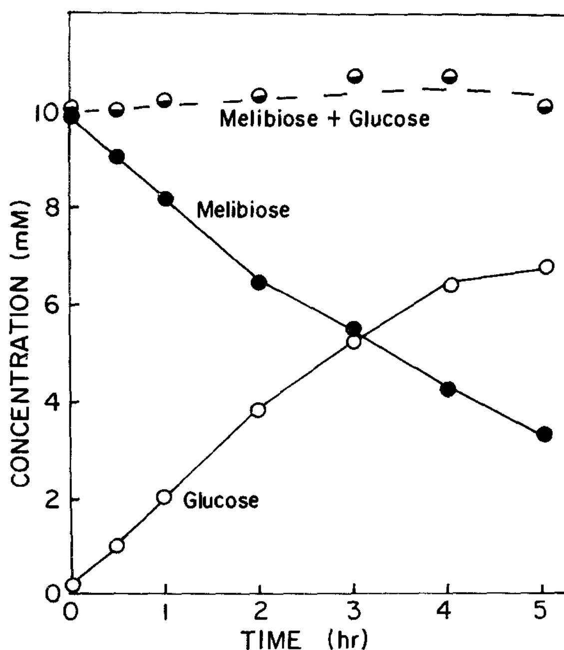

Fig. 1. Melibiose utilization and glucose production in strain GN22. Cells of GN22 (*lacY*$^-$) were grown to logarithmic phase in Medium 63 containing melibiose (10 mM) at 30 °C. Five ml of washed cells (1.8 mg dry wt/ml) were incubated at 30° in Medium 63 containing 100 µg/ml chloramphenicol and 10 mM melibiose. Samples (0.5 ml) were withdrawn at the indicated times and treated with Ba $(OH)_2$ and $ZnSO_4$. The concentrations of both melibiose and glucose were determined as described in *Materials and Methods*. ●, melibiose; ○, glucose; ◒, melibiose + glucose

In order to test the effect of Li^+ on the transport of melibiose, it was essential to develop a method for the measurement of uptake of the disaccharide. The procedure adopted was to estimate melibiose uptake in strain GN22 which lacks glucokinase and enzyme 1. Melibiose enters induced cells of this strain, is split by α-galactosidase, and the glucose quantitatively excreted into the incubation medium. Figure 1 shows that over a period of 5 hr GN22 excreted approximately 1 µmole of glucose for each µmole of melibiose taken up. To be a suitable assay for melibiose transport, it was essential to demonstrate that the uptake step was rate-limiting in the overall process. The membrane carrier of strain GN22 was inactivated by exposure of the cells to 37° for various periods of time and the glucose production measured. In this strain the melibiose carrier, but not the α-galactosidase, is temperature sensitive. If α-galactosidase were rate limiting, significant loss of carrier activity

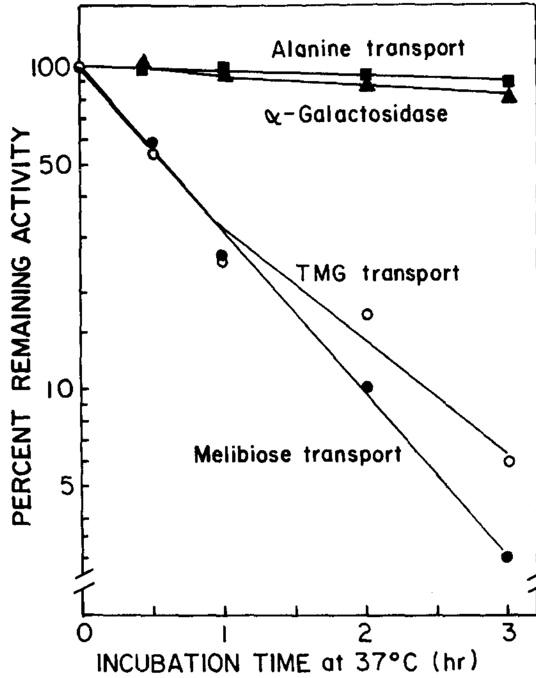

Fig. 2. Inactivation of melibiose transport by heat treatment in GN22. Cells of strain GN22 (*lacY*$^-$) were grown on melibiose at 30 °C. Washed cells (1.2 mg dry wt/ml) were incubated at 37° in Medium 63 containing 100 μg/ml of chloramphenicol. At the indicated times samples were removed and cooled in an ice bath until assayed. A portion of the sample was assayed for melibiose transport at 30°. A second portion was assayed for TMG transport in the presence of 10 mM Li^+-D-lactate at 25 °C. A third portion was assayed for α-galactosidase activity. The last portion was assayed for alanine transport at room temperature. Initial values (100%) were: 12.0 nmoles glucose formed/min/mg dry wt for TMG transport, 19.3 nmoles *p*-nitrophenol formed/min/mg dry wt for α-galactosidase activity, 3.71 nmoles alanine taken up/min/mg dry wt for alanine transport. ●, melibiose transport; ○, TMG transport; ▲, α-galactosidase activity; ■, alanine transport

could occur before a reduction in glucose excretion would be observed. Figure 2 shows that progressive inactivation of the carrier resulted in a comparable loss of glucose excretion. This is consistent with the view that transport is rate-limiting in this assay system. The parallel loss of transport capacity of both TMG and melibiose is consistent with the view that these two sugars are both transported by the same transport system. Alanine transport was measured in this experiment as a control simply to indicate that a temperature-stable transport system was not inactivated under these conditions.

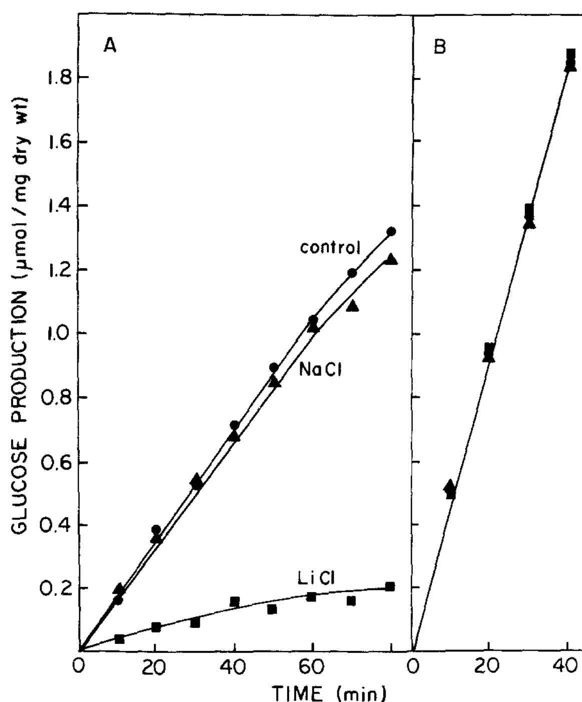

Fig. 3. Inhibition of melibiose entry by Li^+. (*A*): Cells of GN22 (*lacY*$^-$) were grown at 30 °C in Medium 63 plus melibiose. Washed cells (0.82 mg/ml) were preincubated for 10 min at 30° in the presence of 10 mM LiCl (■) or 10 mM NaCl (▲), or in the absence of these salts (●). Melibiose was added to give a final concentration of 10 mM, samples were taken at time intervals, and glucose production was measured. (*B*): Cells of GN2 were grown at 37° in the presence of melibiose (lactose transport system present, melibiose transport system absent). Washed cells were preincubated at 37° for 10 min with or without NaCl or LiCl. Melibiose entry was measured at 37°. Symbols are the same as in *A*

Effect of Li^+ *on Melibiose Transport*

The effect of Li^+ and Na^+ on melibiose transport was tested in GN22 by the glucose excretion method. Figure 3*A* shows that 10 mM Li^+ strongly inhibited glucose production from melibiose. Since Li^+ has no effect on α-galactosidase, this cation must inhibit the transport step. Na^+ has no effect on this transport process under these conditions. The Li^+ inhibition of growth on melibiose, therefore, appears to be due to an inhibition of melibiose uptake into the cell.

The effect of Li^+ on melibiose transport by the lactose transport system was tested in GN2, which possesses a constitutive lactose operon with an intact *Y* gene. The melibiose transport system was inactivated

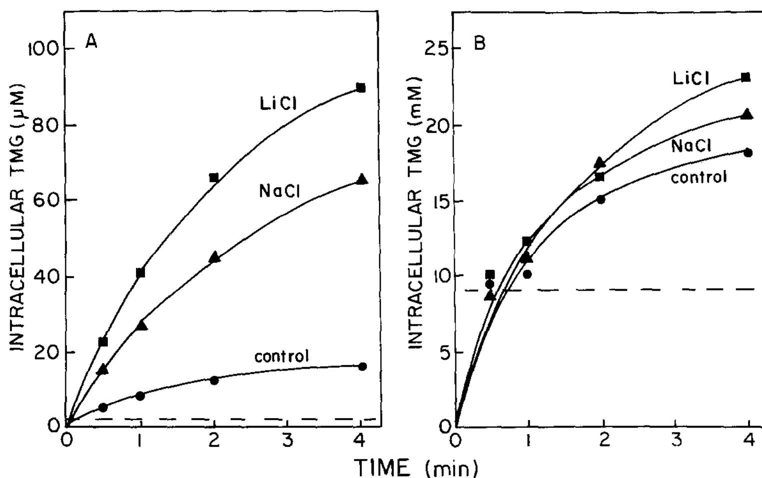

Fig. 4. Effect of Li^+ and Na^+ on TMG uptake in GN22. Cells of GN22 were incubated in MOPS buffer pH 7 and tested for TMG uptake at (*A*) low concentration (2 μM) and (*B*) high concentration (9 mM) of TMG. Symbols: control, ●; +10 mM LiCl, ■; +10 mM NaCl, ▲. Dotted lines show the concentration of TMG in the medium

by growing the cells at 37°. When GN2 was grown on melibiose at 37° the disaccharide enters the cell via the lactose transport system and induces α-galactosidase. Melibiose is then split to galactose which is metabolized and glucose which is excreted. Melibiose transport via the lactose transport system in these cells was not affected by Na^+ or Li^+ (Fig. 3 *B*). This is further evidence that these two ions do not affect α-galactosidase.

Since Li^+ and Na^+ stimulate the transport of TMG mediated by melibiose transport system in several strains of *E. coli* (Tsuchiya *et al.*, 1977; Lopilato *et al.*, 1978), it was important to confirm this observation with strain GN22. Figure 4 shows that Li^+ and Na^+ stimulated TMG uptake with melibiose-induced GN22. When the concentration of TMG was low (2 μM), the stimulation by Li^+ or Na^+ was large. This result is consistent with TMG-Na^+ or TMG-Li^+ cotransport. Li^+ or Na^+ lowered the K_m value for TMG uptake without affecting the V_{max} (Lopilato *et al.*, 1978). In the presence of a high concentration of TMG (9 mM), Li^+ or Na^+ stimulated the uptake only slightly. Thus, in the same cell (GN22) Li^+ stimulated TMG transport and inhibited melibiose transport. Although Na^+ did not have any significant effect on transport at 10 mM melibiose (Fig. 3), Na^+ may affect melibiose transport at low concentrations of melibiose. At the present time this possibility cannot be tested

Table 3. Effect of monvalent cations on melibiose transport system in GN22

Monovalent cations[a]	Melibiose entry		TMG uptake[b]	
	(nmoles glucose/ min/mg dry wt)	(%)	(nmoles/min/mg) dry wt)	(%)
–	10.6	100	2.25	100
KCl	10.5	99	2.16	96
NaCl	9.8	92	6.74	300
LiCl	1.9	18	9.29	413
NH_4Cl	9.8	92	2.07	92
RbCl	9.9	93	2.17	96
CsCl	10.3	97	2.25	100
Choline·Cl	10.0	94	2.31	103

[a] Monovalent cations were added at 10 mM.
[b] The initial rate of TMG uptake was determined at 30 sec.

since a high concentration of melibiose is required for the glucose excretion technique.

The effect of other monovalent cations on melibiose entry and TMG accumulation was tested (Table 3). Li^+ was the only monovalent cation which inhibited the entry of melibiose. On the other hand, Li^+ and Na^+ stimulated TMG uptake. K^+, Na^+, NH_4^+, Rb^+, Cs^+, and choline$^+$ were ineffective.

The effect of Li^+ and Na^+ on the transport of some other galactosides was next investigated. As shown in Fig. 5, Li^+ and Na^+ inhibited the entry of α-PNPG. Na^+, on the other hand, stimulated the entry of β-PNPG. Li^+ had no effect on β-PNPG entry. The transport of raffinose (an α-galactoside consisting of galactose, glucose, and fructose) was stimulated twofold by Na^+ but affected only slightly by Li^+ (Fig. 6).

Are TMG and Melibiose Transported by a Single Carrier?

The question arises whether the transport of melibiose and of TMG take place on the same membrane carrier? Evidence for a single carrier is as follows: (i) TMG transport activity and melibiose transport activity are induced simultaneously by growth in the presence of melibiose at 30° in strain GN22, a mutant lacking the lactose transport system (Table 4); (ii) Both activities are absent in GN22 induced at 37° (Table 4);

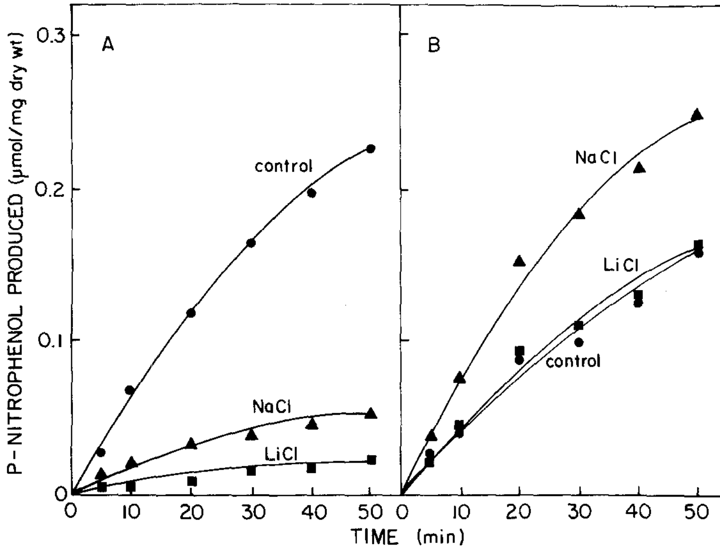

Fig. 5. Effect of Li^+ and Na^+ on PNPG entry in GN22. Melibiose-induced cells of GN22 were incubated in the presence of 0.5 mM α-PNPG or β-PNPG and the production of *p*-nitrophenol measured. (*A*): α-PNPG entry. (*B*): β-PNPG entry. Symbols: control, ●; +10 mM LiCl, ■; +10 mM NaCl, ▲

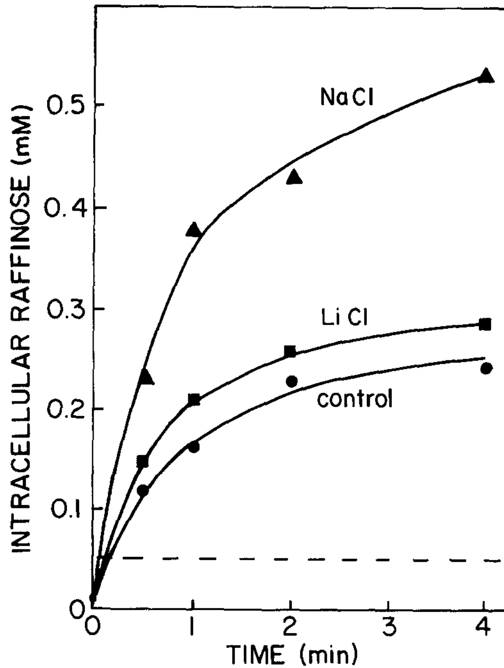

Fig. 6. Effect of Li^+ and Na^+ on the transport of raffinose. Washed cells of GN22 were used for the measurement of raffinose uptake. Symbols: control, ●; +10 mM LiCl, ■; +10 mM NaCl, ▲

Table 4. Effect of temperature on melibiose transport system in GN22 and GN22HA

Strain	Conditions[a]	Melibiose entry		TMG uptake[b]	
		(nmoles glucose/ min/mg dry wt)	(%)	(nmoles/min) mg dry wt)	(%)
GN22	Uninduced	0.0	0	0.1	1
	Induced at 30°	16.8	100	13.7	100
	Induced at 37°	0.4	2	0.3	2
GN22HA	Induced at 30°	22.5	100	10.9	100
	Induced at 37°	15.2	68	8.0	73

[a] Cells were grown on tryptone in the presence or absence of melibiose (10 mM).
[b] Initial rate of TMG uptake was determined at 30 sec.

(iii) The strongest evidence that the two substrates are transported by the same carrier comes from studies of the temperature sensitivity of carrier. When the transport carrier of GN22 was inactivated by incubation of the cells at 37°, TMG accumulation and melibiose uptake were reduced in parallel (Fig. 2). Furthermore, a mutant (GN22HA) possessing a temperature-resistant melibiose carrier was isolated from GN22. When strain GN22HA was grown in the presence of melibiose at 37°, The transport of both TMG and melibiose were found to be present (Table 4).

Li^+ Resistant Mutant

When strain GN22 was incubated in minimal medium containing melibiose plus 10 mM LiCl at 30 °C, no growth was observed after 24 h. After another 1–2 days incubation, growth was observed and Li^+-resistant mutants were isolated. The Li^+-sensitivity of melibiose transport in one such mutant GN22LI is shown in Fig. 7. Transport in GN22LI was much more resistant to Li^+ than its parent GN22. GN22LI does not grow on melibiose at 37°, indicating that the heat sensitivity of melibiose transport system was retained. Li^+ stimulation of TMG transport was retained in this mutant (Fig. 8). In addition, Na^+-stimulation of TMG accumulation was normal. The activities of both the melibiose transport system and the α-galactosidase in GN22LI were about 3 times higher than that of GN22. Four additional Li^+-resistant mutants were tested, and each showed elevated levels of α-galactosidase and of melibiose transport compared with the parental strain.

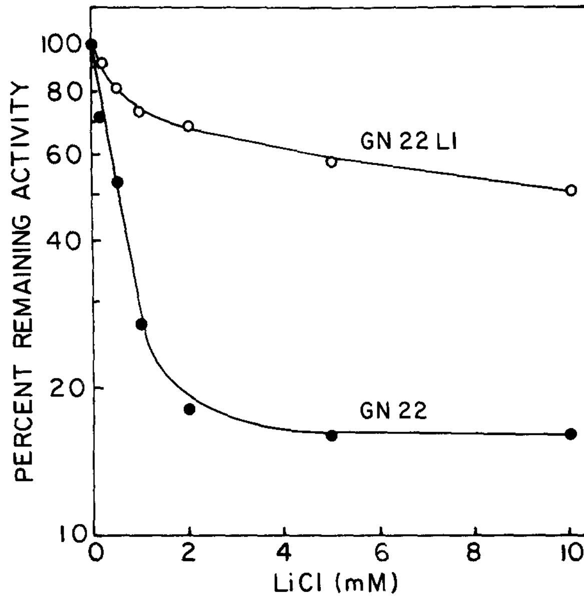

Fig. 7. Effect of Li^+ on melibiose entry in GN22LI and GN22. Washed cells of GN22LI (Li^+-resistant) (○) and at GN22 (Li^+-sensitive) (●) were incubated with LiCl at indicated concentration plus melibiose (10 mM). After 30 min at 30° samples were taken, and glucose production was measured. 100% for GN22LI was 1.09 μmole glucose/mg cells/30 min, and that for GN22 was 0.33 μmole glucose/mg/cells/30 min

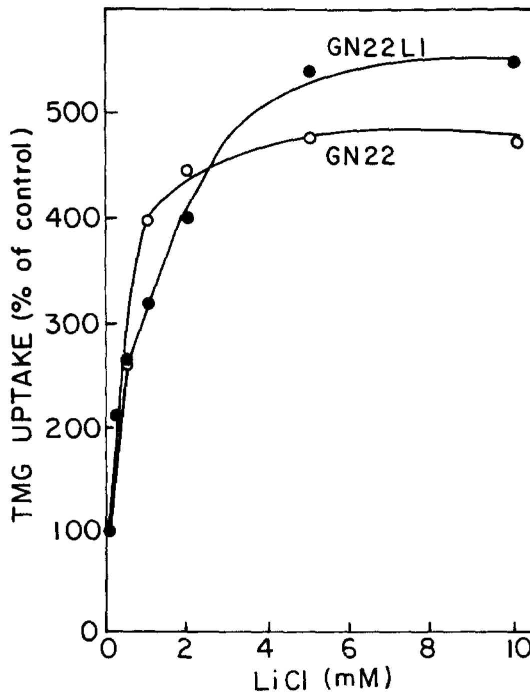

Fig. 8. Effect of Li^+ on TMG uptake in GN22LI and GN22. Cells were incubated in MOPS buffer (pH 7) containing ^{14}C-TMG at a concentration of 0.05 mM. Samples were taken at 30 sec for the measurement of TMG uptake with GN22LI (●) and GN22 (○). 100% for GN22LI was 1.32 mM of intracellular TMG, and that for GN22 was 0.35 mM

Discussion

Previous studies with the melibiose transport system of *E. coli* have shown that Li^+ as well as Na^+ stimulates TMG accumulation (Tsuchiya *et al.*, 1977; Lopilato *et al.*, 1978). An electrochemical potential difference for Li^+ (or Na^+) can drive TMG accumulation by energy-depleted cells of *E. coli*. Similarly, TMG addition can drive Na^+ uptake in energy-depleted cells (Tsuchiya & Wilson, *unpublished observations*). Furthermore, in the presence of Li^+ (or Na^+) the addition of TMG results in proton exit from the cell. Presumably Li^+ (or Na^+) entry with TMG provides a membrane potential (inside positive) which results in H^+ exit. Thus, lithium and sodium appear to be equally effective in TMG cotransport.

The inhibition of melibiose transport by Li^+ was therefore an unexpected finding. The possibility that melibiose and TMG are transported by different systems has been ruled out by several observations. The transport of both substrates is induced by growth of GN22 on melibiose at 30 °C and is absent in GN22 grown at 37 °C. Furthermore, TMG accumulation and melibiose uptake are both inactivated equally by incubating strain GN22 at 37°. In this particular strain the melibiose transport system is temperature-sensitive. In addition a mutant, GN22HA, which grows well on melibiose at 37° possesses transport capacity for both TMG and melibiose. All of these data taken together support the view that both melibiose and TMG are transported by the same membrane carrier.

Three additional sugars were tested for their cation requirements. Both raffinose and β-*p*-nitrophenylgalactoside showed a sodium stimulation of uptake but little or no effect by Li^+. Transport of α-*p*-nitrophenylgalactoside, on the other hand, was inhibited by both Na^+ and Li^+. Thus it would appear that different substrates for the same transport system can be affected differently by sodium and lithium ions.

A lithium-resistant mutant of GN22 was isolated by growth of these cells on melibiose in the presence of 10 mM LiCl. The melibiose transport in this mutant was found to be far less sensitive to the presence of Li^+ than the parental cell. TMG accumulation by this mutant was stimulated by Li^+ in a similar manner to that found in the parent. An interesting aspect of this mutant was that when grown in melibiose it produced approximately three times as much α-galactosidase and TMG transport than the parental cell.

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