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Cation Coupling to Melibiose Transport in *Salmonella typhimurium*

SUMIKO NIIYA, YOSHINORI MORIYAMA, MASAMITSU FUTAI, AND TOMOFUSA TSUCHIYA*

Department of Microbiology, Faculty of Pharmaceutical Sciences, Okayama University, Tsushima-naka, Okayama 700, Japan

Melibiose transport in *Salmonella typhimurium* was investigated. Radioactive melibiose was prepared and the melibiose transport system was characterized. Na^+ and Li^+ stimulated transport of melibiose by lowering the K_m value without affecting the V_{max} value; K_m values were 0.50 mM in the absence of Na^+ or Li^+ and 0.12 mM in the presence of 10 mM NaCl or 10 mM LiCl. The V_{max} value was 140 nmol/min per mg of protein. Melibiose was a much more effective substrate than methyl-β-thiogalactoside. An Na^+-melibiose cotransport mechanism was suggested by three types of experiments. First, the influx of Na^+ induced by melibiose influx was observed with melibiose-induced cells. Second, the efflux of H^+ induced by melibiose influx was observed only in the presence of Na^+ or Li^+, demonstrating the absence of H^+-melibiose cotransport. Third, either an artificially imposed Na^+ gradient or membrane potential could drive melibiose uptake in cells. Formation of an Na^+ gradient in *S. typhimurium* was shown to be coupled to H^+ by three methods. First, uncoupler-sensitive extrusion of Na^+ was energized by respiration or glycolysis. Second, efflux of H^+ induced by Na^+ influx was detected. Third, a change in the pH gradient was elicited by imposing an Na^+ gradient in energized membrane vesicles. Thus, it is concluded that the mechanism for Na^+ extrusion is an Na^+/H^+ antiport. The Na^+/H^+ antiporter is a transformer which converts an electrochemical H^+ gradient to an Na^+ gradient, which then drives melibiose transport. Li^+ was inhibitory for the growth of cells when melibiose was the sole carbon source, even though Li^+ stimulated melibiose transport. This suggests that high intracellular Li^+ may be harmful.

Sodium ion-substrate cotransport is generally observed in the active transport processes of animal membrane systems (3), whereas H^+ is frequently associated with active transport in microorganisms. In 1971, Stock and Roseman (12) suggested the possibility of Na^+-methyl-β-D-thiogalactopyranoside (TMG) cotransport in *Salmonella typhimurium*. We subsequently found that TMG is transported by a cotransport mechanism with Na^+ via the melibiose transport system of *Escherichia coli* (4, 18, 22). Two lines of evidence supported this finding. First, an artificially imposed electrochemical gradient of Na^+ elicited uptake of TMG in energy-starved cells (18). Second, the influx of TMG down its chemical gradient elicited influx of Na^+ in nonglycolysing cells under anaerobic condition (22). These results were obtained only with melibiose-induced cells. Since the melibiose transport system is inducible, these observations are consistent with the idea that the melibiose carrier catalyzes Na^+-TMG cotransport. The Na^+-glutamate cotransport mechanism also occurs in *E. coli* (7, 16). A similar Na^+-substrate cotransport system also exists in *Halobacterium halobium* (6). The existence of Na^+-substrate cotransport in microbial systems is important from an evolutionary point of view. Both H^+ and Na^+ were found to be coupling ions for melibiose transport via the melibiose carrier. Melibiose is also transported via the lactose transport system. In this case, however, H^+ appears to be the sole coupling ion (22).

Characterization of the melibiose transport system in *S. typhimurium* was performed by Tokuda and Kaback (15), using radioactive TMG as a transport substrate. TMG is not a natural substrate for this system, whereas melibiose is. Using [^{3}H]melibiose, we characterized melibiose transport in *S. typhimurium*. We determined the kinetic parameters of this system and investigated the specificity of cation coupling to melibiose transport in *S. typhimurium*.

MATERIALS AND METHODS

Bacterial strain and growth. *S. typhimurium* LJ3 was generously supplied by M. H. Saier, University of California. This strain lacks α-galactosidase activity (9). Cells were grown in minimal salt medium (14) supplemented with 1% tryptone (Difco) and 1

mM melibiose. Na^+ salts were replaced with K^+ salts in the medium. Cells were harvested after approximately three to four doublings.

Preparation of [^{3}H]melibiose. [^{3}H]melibiose was prepared from [^{3}H]raffinose as described previously (13). Briefly, [^{3}H]raffinose was cleaved by invertase to give melibiose and fructose. The mixture of uncleaved raffinose, melibiose, and fructose was fractionated by application to a column of Sephadex G-15. Fractions containing melibiose were pooled and lyophilized. Radioactive melibiose thus obtained was pure as judged by paper chromatography (13).

Transport assays. Cells were washed twice in 100 mM morpholinopropanesulfonic acid (MOPS) buffer adjusted to pH 7.0 with Tris and suspended in the same solution. [^{3}H]melibiose or [^{14}C]TMG was added to cells (0.25 to 0.30 mg of protein per ml) suspended in 100 mM MOPS-Tris buffer, pH 7.0, to give a final concentration of 50 μM. Samples (200 μl) were taken at intervals, filtered on membrane filters (0.45-μm pore size; Toyo Co.), and washed with 100 mM MOPS-Tris buffer, pH 7.0, at room temperature (18 to 20°C). The filters were dried and counted. In the experiments described in Fig. 2, 100 mM potassium phosphate buffer, pH 7.0, was used throughout instead of MOPS-Tris buffer. Various concentrations of NaCl and LiCl were added to the assay mixture when appropriate. In the experiments given in Fig. 5, energy-starved cells were prepared as described by Berger (2), and transport energized by an artificial driving force was measured as described previously (4, 18).

Measurement of Na^+ flux and H^+ flux. Na^+ influx induced by sugar influx down its concentration gradient was measured as described previously (22), using an Na^+ electrode. Extrusion of Na^+ energized by respiration or glycolysis was measured as described previously (20), with cells grown as described above. H^+ flux was measured as described previously (18, 19), using an H^+ electrode.

Measurement of fluorescence quenching. Fluorescence assays with everted membrane vesicles prepared as described previously (19) were performed with a Hitachi fluorometer. 9-Aminoacridine was used as a fluorescent probe for measurement of the pH gradient, as described by Schuldiner and Fishkes (10). Assay conditions were as described previously (19).

Protein assay. Protein contents were determined by the method of Lowry et al. (5).

Chemicals. Melibiose, methyl-α- and β-galactopyranosides, TMG, and invertase (EC 3.2.1.26) were purchased from Sigma Chemical Co. [*G*-^{3}H]raffinose and [*methyl*-^{14}C]TMG were from New England Nuclear Corp. Carbonyl cyanide-*m*-chlorophenyl hydrazone was from Calbiochem. All other chemicals were of reagent grade and were obtained from commercial sources.

RESULTS

Effect of cations on melibiose transport. *S. typhimurium* LJ3 is a mutant lacking α-galactosidase and cannot utilize melibiose (9). There is only one system known which transports melibiose in *S. typhimurium*, namely, the melibiose transport system. Thus, this strain is suitable for the measurement of melibiose transport. Recently, we have developed a method for the preparation of radioactive melibiose, making it possible to measure transport of melibiose itself (13). Na^+ greatly stimulated melibiose transport in whole cells of strain LJ3 (Fig. 1A). A similar stimulatory effect on melibiose transport by Na^+ was observed in *E. coli* (13). Li^+ also stimulated melibiose transport in LJ3, whereas K^+ and other cations had no effect. Higher stimulation of melibiose transport was obtained with Li^+ than with Na^+. The optimal concentration of Na^+ and Li^+ for the stimulation of melibiose transport was around 10 mM (data not shown). TMG is a substrate of this transport system and has been used exclusively to measure its activity. We compared the activity of this system for melibiose and TMG. At the same concentration of both substrates, melibiose transport was much higher than that of TMG. As reported by Stock and Roseman (12) and as shown in Fig. 1B, both Na^+ and Li^+ stimulated TMG transport. As with *E. coli* (4, 18), Li^+ stimulated TMG transport better than Na^+. The kinetic parameters of melibiose transport were determined (Fig. 2). Na^+ or Li^+ lowered the K_m value of melibiose transport without affecting the V_{max}, which was estimated to be 140 nmol/min per mg of protein. The K_m was 0.50 mM in the absence of Na^+ and Li^+ and 0.12 mM in the presence of 10 mM Na^+ or Li^+. The K_m of TMG transport was reported to be 0.8 mM in the presence of 20 mM LiCl in membrane vesicles of *S. typhimurium* (15).

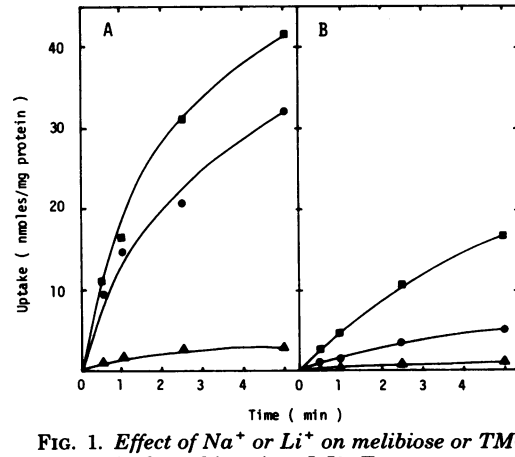

FIG. 1. *Effect of Na^+ or Li^+ on melibiose or TMG transport in S. typhimurium LJ3. Transport assays were performed as described in the text. Cells were incubated in the assay medium containing (A) [^{3}H]-melibiose (50 μM) or (B) [^{14}C]TMG (50 μM). Symbols: Control (▲); NaCl (●) or LiCl (■), 10 mM, was added to the assay mixture, and the mixture was incubated for 10 min before initiation of the reaction.*

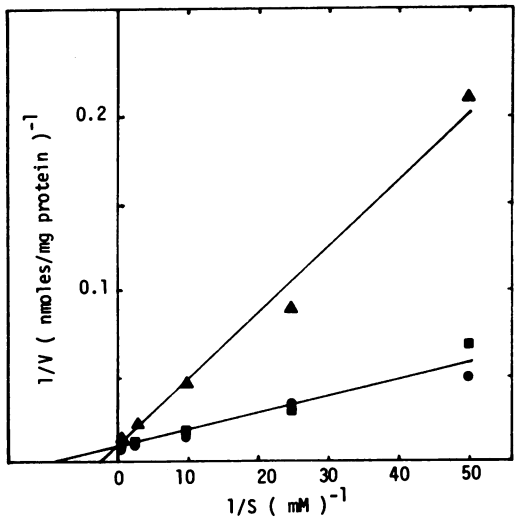

FIG. 2. *Determination of kinetic parameters of melibiose transport. Cells were added to the assay medium containing various concentrations of [^{3}H]-melibiose. Initial rates of melibiose uptake were measured at 30 s. Symbols: Control (▲); 10 mM NaCl (●) or 10 mM LiCl (■) was added to the assay mixture.*

Na^+ (Li^+)-melibiose cotransport. Several lines of evidence indicate that melibiose is taken up together with Na^+ by cotransport in *E. coli* (4, 18, 22). We tested the possibility of Na^+-melibiose cotransport in *S. typhimurium* by three methods. At the same time, cation specificity for melibiose transport was determined. The first procedure used was to detect Na^+ fluxes across the cell membrane after the imposition of an inwardly directed melibiose gradient. After the addition of 5 mM melibiose to the cell suspension under anaerobic conditions, Na^+ influx into cells was observed (Fig. 3). This observation indicates that melibiose entry into cells down its concentration gradient elicited simultaneous uptake of Na^+. These observations could not be obtained with melibiose-uninduced cells, which lack the melibiose transport system, suggesting that Na^+ influx took place through the melibiose carrier. Methyl-α-galactoside also caused Na^+ influx, but the rate and extent of Na^+ influx elicited by methyl-α-galactoside were less than that elicited by melibiose. On the other hand, TMG caused very little movement of Na^+ into cells. This suggests that the efficiency of Na^+-coupled transport of TMG is not high compared with Na^+-coupled transport of melibiose or methyl-α-galactoside. Methyl-β-galactoside did not cause a significant influx of Na^+. Methyl-β-galactoside either is not a substrate for the melibiose transport system or cannot couple to Na^+ in the transport process.

The second procedure was to detect H^+ flux induced by cation-coupled transport of the substrate. If a cation-sugar cotransport process exists, then such a process should be electrogenic. In *E. coli* Na^+-TMG cotransport elicited H^+ efflux (22). This H^+ movement was inhibited by the permeable anion SCN^-, suggesting that Na^+-TMG influx generated membrane potential (interior positive) and that H^+ movement toward the negative side occurred in response. The addition of melibiose to a cell suspension in the absence of Na^+ or Li^+ did not cause H^+ flux, suggesting that H^+-melibiose cotransport does not exist in *S. typhimurium* (Fig. 4). If H^+-melibiose cotransport exists, then H^+ influx should have been observed. Addition of SCN^- to the assay system in the absence of Na^+ or Li^+ did not have any significant effect. Thus, the possibility of H^+-melibiose cotransport, which exists in *E. coli* (21, 22), was eliminated. Addition of melibiose to the cell suspension in the presence of either Na^+ or Li^+ caused efflux of H^+ (Fig. 4). Thus, the notion of Na^+ (Li^+)-melibiose cotransport was supported. Similarly, the data suggest both Na^+ (Li^+)-TMG and Na^+ (Li^+)-methyl-α-galactoside cotransport. On the other hand, neither H^+-TMG nor H^+-methyl-α-galactoside cotransport seems to exist.

Third, we tested whether or not an artificially imposed electrochemical gradient of Na^+ could support melibiose uptake in energy-starved cells. Melibiose transport was supported by such an electrochemical potential difference of Na^+ across the cell membrane (Fig. 5). Either the

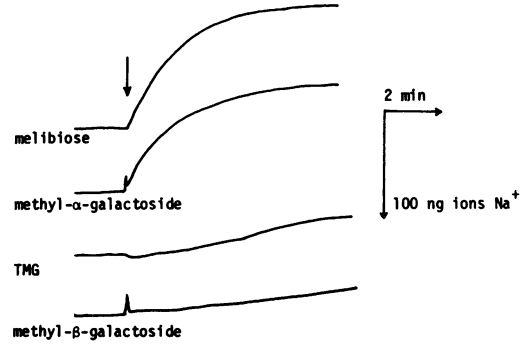

FIG. 3. *Extracellular pNa changes on addition of various galactosides to cell suspensions. LJ3 cells were suspended in medium containing 100 mM Tricine-tetramethylammonium hydroxide, pH 8.0, and 25 μM NaCl and incubated under anaerobic conditions. Various sugars were added to strain LJ3 at the time indicated by the arrow to give a final concentration of 5 mM. An upward deflection of the tracing indicates a decrease in Na^+ concentration in the incubation medium. The abscissa is time; the ordinate is Na^+ concentration. pNa, $-Log_{10}$ (sodium concentration).*

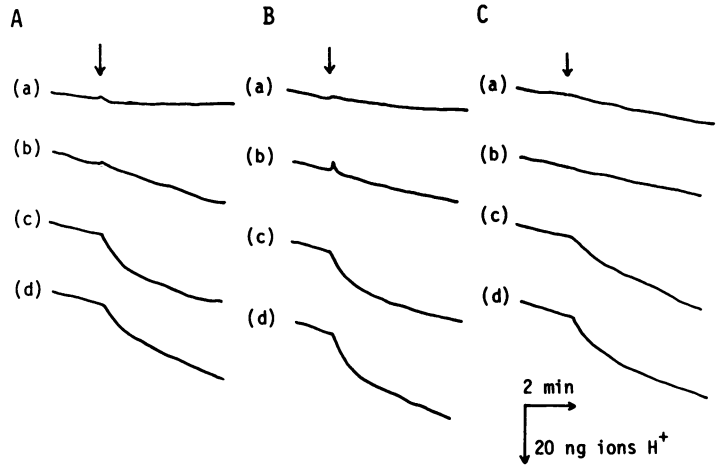

FIG. 4. *Effect of cations on proton movement induced by addition of galactosides. Anaerobic cells of strain LJ3 suspended in 120 mM KCl were incubated in the presence of (a) control, (b) 25 mM KSCN, (c) 10 mM NaCl, (d) 10 mM LiCl. At the time indicated by the arrows, melibiose (A), methyl-α-galactoside (B), or TMG (C) was added to 5 mM. A downward deflection indicates a decrease in the pH of the medium.*

chemical or the electrical component alone also drove melibiose transport, but to a lesser extent (data not shown). Thus, electrogenic Na^+-melibiose cotransport was suggested by three different methods.

Establishment of the Na^+ gradient. To energize melibiose transport in *S. typhimurium*, Na^+ must be extruded in some way. The primary process in membrane energization is proton extrusion by the electron transport chain or the H^+-translocating ATPase (8). In *E. coli* it has been shown that the electrochemical potential difference of H^+ across the cell membrane thus established can be converted to that of Na^+ by means of the Na^+/H^+ antiporter (1, 10, 20, 23). The electrochemical gradient of Na^+ then drives Na^+-coupled transport in this organism. It is likely that the same mechanism operates for the establishment of the Na^+ gradient in *S. typhimurium*, because these two organisms are related. We investigated this plausible mechanism of Na^+ extrusion in *S. typhimurium*. Previously we reported a procedure for the direct measurement of Na^+ transport in whole cells, using an Na^+ electrode (20). With this technique, we observed Na^+ extrusion from cells of *S. typhimurium* energized by respiration or glycolysis (Fig. 6). This extrusion of Na^+ was inhibited by carbonyl cyanide-*m*-chlorophenyl hydrazone, indicating the participation of the electrochemical H^+ gradient in Na^+ extrusion. The second procedure used was detection of H^+ efflux after an inwardly directed Na^+ gradient was imposed, as originally reported by West and Mitchell (23). Efflux of H^+ was detected after addition of Na^+ or Li^+ to cell suspensions under anaerobic conditions (Fig. 7). The addition of Li^+ caused more efflux of H^+ than did Na^+ addition. Addition of Na^+ in the presence of Li^+ (or vice versa) did not cause H^+ efflux (data not shown). It should be noted that the rapid initial response is due to an effect of Na^+ (Li^+) on the glass electrode, as pointed out by West and Mitchell (23). Na^+ inflow caused effective outflow of H^+ even in the presence of SCN^- (data not shown). Thus, it is concluded that Na^+ influx and H^+ efflux are not electrically coupled, but are chemically coupled by an Na^+/H^+ antiport mechanism. To confirm the Na^+/H^+ antiport mechanism, we tested the effect of Na^+ and Li^+ on the H^+ gradient by measuring fluorescence quenching of 9-aminoacridine. After addition of succinate, quenching of 9-aminoacridine took place, indicating formation of an H^+ gradient across everted membrane vesicles. As originally reported by Schuldiner and Fishkes (10) in *E. coli*, both Na^+ and Li^+ partially collapsed the H^+ gradient established across everted membrane vesicles of *S. typhimurium* (Fig. 8). Addition of Li^+ in the presence of Na^+ (or vice versa) did not collapse the pH gradient further. All of these observations indicate that Na^+ and Li^+ are extruded by an antiport mechanism with H^+ and that Na^+ and Li^+ share a common transport system, as in the case of *E. coli* (10). Concentrations of the salts giving half-maximal collapse of the H^+ gradient were 4.5 and 0.38 mM for NaCl and LiCl, respectively. Because of a technical problem, we could not measure the rate of collapse of the H^+ gradient. Although we do not know the actual affinity of these two ions, Li^+ seems to have a much higher affinity for this antiporter.

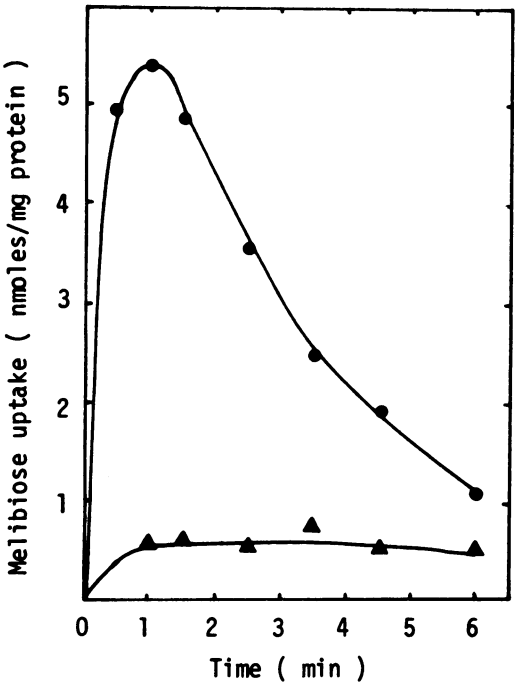

FIG. 5. *Melibiose accumulation induced by an artificial driving force in energy-depleted cells. A membrane potential ($\Delta\psi$; inside negative) was imposed as a diffusion potential of H^+ (inside acid) in the presence of carbonyl cyanide-m-chlorophenyl hydrazone (CCCP). At the same time an inwardly directed chemical gradient of Na^+ (ΔpNa) was imposed by the addition of Na^+ to the external medium. Energy-depleted cells were divided into two samples. One was washed with buffer containing 50 mM potassium phosphate (pH 8.0) and 50 mM Na_2SO_4, and the other was washed with buffer containing 50 mM potassium phosphate (pH 6.0) and 50 mM K_2SO_4. At the end of the final wash CCCP and KCN were added to each of two samples to give final concentrations of 5 μM and 2 mM, respectively. Ten minutes after addition of CCCP and KCN, cells were diluted 1:20 into a solution containing 50 mM potassium phosphate (pH 8.0), 50 mM Na_2SO_4, 5 μM CCCP, 2 mM KCN, and 50 μM [^{3}H]melibiose. Samples were taken at intervals. Symbols: Control (no driving force) (▲); $\Delta\psi$ + ΔpNa (●).*

Effect of Li^+ on growth of cells on melibiose. As reported previously (4, 18), Li^+ stimulated TMG transport via the melibiose transport system in *E. coli*. On the other hand, Li^+ inhibited melibiose transport via the same system (13, 17, 22). Thus, Li^+ strongly inhibited the growth of *E. coli* cells, which lack the lactose transport system when melibiose is added as a sole source of carbon (17). Since Li^+ does not inhibit melibiose transport mediated by the lactose transport system, Li^+ does not inhibit the growth of wild-type *E. coli* cells even if melibiose is the sole carbon source. *S. typhimurium* does not possess the lactose system. It is likely that melibiose is taken up solely via the melibiose system in this organism. We tested the effect of Li^+ on the growth of *S. typhimurium* on melibiose. Since Li^+ did not inhibit melibiose transport (Fig. 1), Li^+ would not be expected to inhibit growth. Surprisingly, however, we observed considerable inhibition of the growth by Li^+ (Fig. 9). Na^+ did not affect the growth, as observed in *E. coli* (17). The media used in this study contained several microequivalents of Na^+ per liter. Thus, such a low concentration of Na^+ may be sufficient to support melibiose transport.

DISCUSSION

Most of the studies on the melibiose transport system of *S. typhimurium* were performed with TMG as a substrate (12, 15). Since radioactive melibiose was not available, it was difficult to investigate several important aspects of the active transport of melibiose. Since melibiose is a natural substrate, it would be desirable to test melibiose transport directly. As reported previously (13), we have developed a method for the preparation of radioactive melibiose. Using this substrate, we characterized the melibiose transport system of *S. typhimurium*. Transport activity for melibiose in *S. typhimurium* is very high. The K_m of the transport was severalfold lower and the V_{max} was severalfold higher than those of *E. coli*. Melibiose seems to be a better substrate than TMG. The K_m of melibiose transport (0.12 to 0.50 mM) is lower than that of TMG (0.8 to 3.8 mM) (15). It should be pointed out that the value for melibiose was obtained with whole cells and that for TMG was obtained with membrane vesicles. Still, as shown in Fig. 1 and 3, we observed more transport of melibiose and more transport of the coupling ion with melibiose than with TMG at the same concentrations of melibiose and TMG, using whole cells of *S. typhimurium*.

The demonstration of Na^+-melibiose cotransport is consistent with the concept of Na^+-TMG cotransport as reported by Stock and Roseman (12) and Tokuda and Kaback (15). Stock and Roseman (12) observed that Na^+ or Li^+ stimulated TMG uptake and that the intracellular Na^+ concentration was higher in the presence of TMG than in its absence. From these results they suggested the possibility of Na^+-TMG cotransport. Later, Tokuda and Kaback (15) observed TMG-dependent Na^+ uptake and Na^+-dependent TMG uptake in membrane vesicles induced by a membrane potential. Approximately equimolar amounts of TMG and Na^+

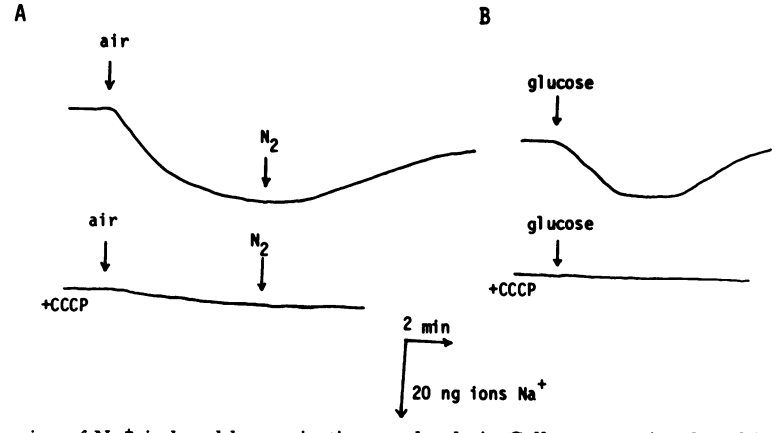

FIG. 6. *Extrusion of Na^+ induced by respiration or glycolysis. Cells were preincubated in 0.1 M Tricine-tetramethylammonium hydroxide buffer (pH 8.0) containing 5 μM NaCl under anaerobic conditions. Carbonyl cyanide-m-chlorophenyl hydrazone (CCCP; 20 μM) was added where indicated. (A) At the time indicated, water-saturated air was introduced into the assay vessel. After a few minutes, air was replaced with N_2 gas. (B) Glucose (final concentration, 0.1 mM) was added to the assay mixture at the points indicated under anaerobic conditions. A downward deflection indicates an increase in Na^+ concentration in the external medium.*

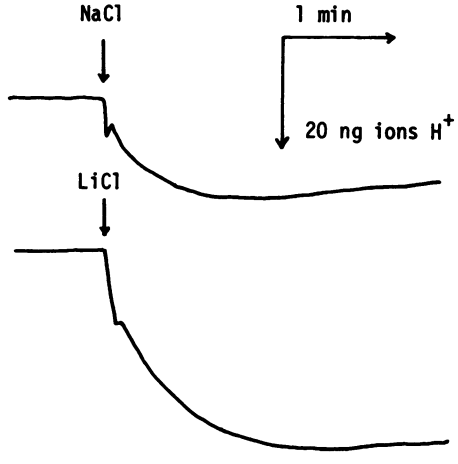

FIG. 7. *H^+ efflux induced by Na^+ or Li^+ influx. pH changes induced by the addition of NaCl or LiCl to the assay mixture were measured with an H^+ electrode as described by West and Mitchell (23). The electrode vessel contained, in 2.0 ml, 120 mM KCl and 2 mg of cell protein. The suspensions were equilibrated anaerobically at pH 7.4. A 20-μl amount of an anaerobic solution of 1 M NaCl or 1 M LiCl was injected at the arrow. A downward deflection represents a decrease in the pH of the medium, indicating H^+ efflux.*

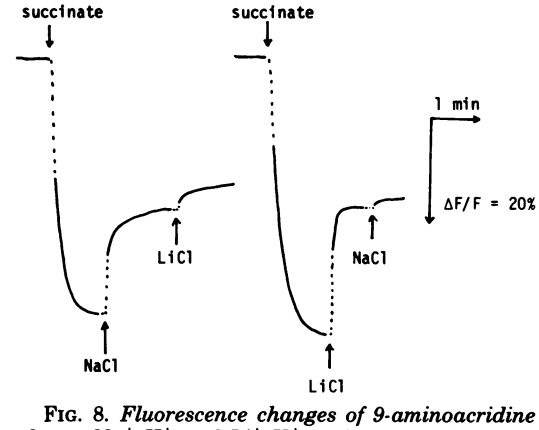

FIG. 8. *Fluorescence changes of 9-aminoacridine due to Na^+-H^+ and Li^+-H^+ antiport activity. Fluorescence quenching and partial reversal were measured as described in the text. Succinate (K^+ salt), NaCl, and LiCl were added to the assay mixture, at the points indicated by arrows, to 5, 10, and 10 mM, respectively. A downward deflection indicates a decrease in fluorescence intensity, reflecting the establishment of a ΔpH, acidic interior.*

were taken up under the conditions used by those authors. We used two direct methods and one indirect method to demonstrate Na^+-melibiose cotransport, as described in Results. We also confirmed Na^+-TMG cotransport by using similar procedures. We do not know the stoichiometry of melibiose to Na^+ yet. Quantitative analysis of Na^+-melibiose cotransport is now in progress.

Versatility in cation coupling was demonstrated in the melibiose transport system of *E. coli* (22). Na^+ or H^+, but not Li^+, could couple to melibiose transport. Na^+ or Li^+, but not H^+, could couple to TMG transport. All three cations could couple to methyl-α-galactoside transport. We tested whether such versatility in cation

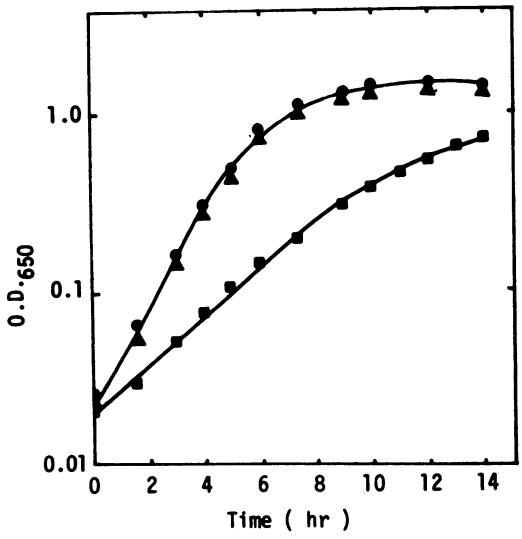

FIG. 9. *Effect of Li^+ on the growth of S. typhimurium LT2 on melibiose. Cells were grown aerobically at 37°C in minimal medium supplemented with 10 mM melibiose. Growth was monitored in terms of absorbance at 650 nm ($O.D._{650}$). Symbols: Control (▲); NaCl (●) or LiCl (■) was added to the medium to 10 mM.*

coupling in the melibiose transport system exists in *S. typhimurium*. Melibiose, TMG, and methyl-α-galactoside showed same cation specificity; namely, these three sugars appear to be cotransported with either Na^+ or Li^+, but not with H^+ (Fig. 3 and 4). The relative affinities of Na^+ and Li^+ have not yet been evaluated. The ability of melibiose to utilize both H^+ and Na^+ for cotransport in *E. coli* is interesting in the context of the evolution of cation coupling to solute transport. In bacterial cells the cotransport mechanism frequently utilizes H^+, whereas Na^+ is utilized in animal cells. Wilson and Maloney (24) postulated that during evolution the proton currency for membrane energetics evolved to that of Na^+. Melibiose transport in *E. coli* may represent an example of the transition in evolution from the H^+ currency to the Na^+ currency. Melibiose transport in *S. typhimurium* cannot utilize H^+ as a coupling ion, although *S. typhimurium* is closely related to *E. coli*. It would be interesting to test the versatility in cation coupling in the cotransport process in many organisms from an evolutional point of view.

Establishment of an Na^+ gradient in *S. typhimurium* is necessary to energize melibiose transport. As in *E. coli*, an Na^+/H^+ antiporter was shown to mediate Na^+ extrusion from cells. Li^+ shares a common carrier with Na^+. Apparently, Li^+ possesses a higher affinity for the carrier than does Na^+. Although we could not compare the velocity of Na^+ and Li^+ extrusion, it seems that Li^+ is extruded more efficiently than Na^+, judging from the magnitude of changes in 9-aminoacridine fluorescence quenching caused by Na^+ and Li^+.

Li^+ was inhibitory for the growth of cells when melibiose was supplied as a sole source of carbon, although Li^+ stimulated melibiose transport. One possible explanation for this puzzling finding would be that intracellular Li^+ at certain concentrations is poisonous. The intracellular Li^+ concentration is determined by a balance between extrusion and influx. When Li^+-melibiose cotransport functions, much Li^+ enters into cells together with melibiose. Thus, the intracellular Li^+ concentration may reach a level unfavorable for some biological processes of cells.

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