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Effects of Sodium and Lithium Ions on the Potassium Ion Transport Systems of *Escherichia coli*†

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ABSTRACT: The effects of the cations choline, Li^+, and Na^+ on the TrkA and Kdp K^+ transport systems in *Escherichia coli* were studied by observing the accumulation of $^{204}Tl^+$ and K^+. Tl^+ uptake via the TrkA system was stimulated by Na^+ but not Li^+ when compared to choline. A similar effect was observed for K^+ transport via the TrkA system. On the other hand, Tl^+ uptake via the Kdp system was stimulated more by Li^+ than by Na^+ when compared to choline. In addition, Li^+ enhanced the effectiveness of Rb^+ as an inhibitor of Tl^+ uptake via the Kdp system. Na^+, however, was a more effective stimulator of K^+ transport via the Kdp system than Li^+. We suggest that Na^+ may be involved in the mechanisms of K^+ transport via the TrkA and Kdp systems in *E. coli*.

The Na^+/K^+-ATPase of animal cells couples the accumulation of K^+ and the extrusion of Na^+ to hydrolysis of ATP, allowing the cells to maintain intracellular levels of K^+ higher than those found outside (Glynn & Karlish, 1975). In *Escherichia coli* four K^+ uptake systems have been described (Rhoads et al., 1976). Of those, the TrkA and Kdp systems are quantitatively the most important. The other two systems, TrkD and TrkF, are quantitatively minor (Rhoads & Epstein, 1978). The constitutive TrkA system, a low-affinity, high-velocity system, is responsible for the majority of K^+ uptake by wild type strains growing in the presence of millimolar or higher levels of K^+. It requires both ATP and a protonmotive force for activity (Rhoads & Epstein, 1977). The high-affinity repressible Kdp system allows cells to grow at K^+ concentrations as low as 1 μM and is ATP driven (Rhoads & Epstein, 1977). Three structural proteins of the Kdp system have been identified, and two of them have molecular weights similar to those of the two subunits of animal Na^+/K^+-ATPase (Laimins et al., 1978). According to Laimins et al. (1978), however, one difference between the two ATPases is that the Kdp system did not appear to require Na^+.

We have previously shown that *E. coli* accumulates $^{204}Tl^+$ via the TrkA and Kdp systems and proposed that $^{204}Tl^+$ could be used as a probe in the study of such systems (Damper et al., 1979). Some initial observations during that work led us to study the effects of several monovalent cations on Tl^+ and K^+ accumulation via the TrkA and Kdp systems. We show here that Na^+ stimulates the transport of both Tl^+ and K^+ via each of these systems.

Experimental Procedure

Bacterial Strains. Two strains of *E. coli* K-12 were used. TK1001 (*trkA*$^+$, *kdp*$^-$, *trkD*$^-$) (Rhoads et al., 1976) was used

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to study the TrkA system, and TK2240 (*kdp*$^+$, *trkA*$^-$, *trkD*$^-$) (Damper et al., 1979) was used to study the Kdp system.

Growth of Cells. For $^{204}Tl^+$ transport experiments, TK1001 was grown overnight at 37 °C in buffer A [the minimal salt medium of Tanaka et al. (1967) containing 54 mM glycerol as the carbon source]. Cells were then transferred to buffer B (buffer A in which choline replaced Na^+ and K^+), supplemented with 25 mM KCl, and allowed to grow for several hours to an OD_{600} of ~1. TK2240 was grown overnight at 37 °C in buffer B supplemented with sufficient buffer A from the inoculum to give 1.4 mM Na^+ and 2.6 mM K^+, transferred to buffer B, and grown for an additional 4 h to derepress the Kdp system. Cells were washed 3 times with buffer C [20 mM Mops (morpholinopropanesulfonic acid) and 135 mM choline chloride, adjusted to pH 7.2 with choline base], suspended in this buffer at ~15 mg of protein per mL, and kept at room temperature until use within 5 h.

For K^+ transport experiments, TK1001 was grown in buffer A. TK2240 was grown overnight in buffer A in which all but 10 mM K^+ was replaced with Na^+ and derepressed by transferring the cells to buffer A in which Na^+ replaced K^+. After being washed once in buffer C, the cells were incubated at 37 °C for 30 min in 10 mM 2,4-dinitrophenol, 20 mM Mops, and 135 mM NaCl (pH 7.2) to deplete the internal K^+, washed 3 times with buffer C, suspended in this buffer at ~30 mg of protein per mL, and kept at room temperature until use within 5 h.

Transport Assays. For measurement of $^{204}Tl^+$ uptake, cells were diluted to 100–200 μg of protein per mL in the assay buffer. The choline assay buffer contained 20 mM Mops, 28 mM glucose, and 135 mM choline chloride with the pH adjusted to 7.2 with choline base. The Na^+ and Li^+ assay buffers were similar except for the replacement of choline by Na^+ and Li^+, respectively. Variations in the concentration of Na^+ and Li^+ in the assay buffer were made by mixing Na^+ or Li^+ assay buffers with choline assay buffer. After incubation of the cells in the assay buffer at room temperature (23 °C) for 5 min, Tl^+ uptake was initiated by adding $^{204}Tl^+$ to 10 μM and 0.5 μCi/mL. In inhibition experiments, KCl or RbCl was added ~5 s prior to the $^{204}Tl^+$. Samples of 0.19 mL were removed at intervals, collected on 0.45-μm pore size nitrocellulose filters (Matheson-Higgins Co., Inc., Woburn, MA), washed with 5 mL of 10 mM Mops containing 200 mM choline chloride and adjusted to pH 7.2 with choline base, dried, and counted.

For measurement of K^+ uptake, cells were diluted to 40–60 μg of protein per mL in the assay buffer (see above) and incubated at 23 °C for 5 min. Uptake was initiated by adding KCl to 38.5 mM. Samples (5 mL) were removed at intervals, filtered, and washed as above. The filters were allowed to dry overnight, treated with 50 μL of concentrated HNO_3, and extracted with 0.95 mL of a $LiNO_3$ diluent for K^+ analysis by flame photometry.

Protein was measured by a modification (Peterson, 1977) of the method of Lowry et al. (1951). Intracellular ion concentrations were calculated by using a value for the internal volume of 2.26 μL per mg of cell protein. In measurements of Tl^+ uptake, intracellular concentrations approaching 10 mM were sometimes observed. At this level some precipitation of Tl^+ salts might occur.

Results

TrkA System. Cells incubated in an Na^+ buffer accumulate much higher levels of $^{204}Tl^+$ via the TrkA system than they do when Na^+ is replaced by choline or Li^+ (Figure 1). There seems to be some initial uptake of Tl^+ in the choline buffer, but the concentration ratio remains fairly constant after ~5 min. Tl^+ uptake in a Li^+ buffer is even slower than in the choline buffer and only reaches a concentration ratio similar to that observed in the choline buffer after ~30 min. In other experiments (not shown) Li^+ appeared to inhibit slightly the Na^+ stimulation of Tl^+ uptake.

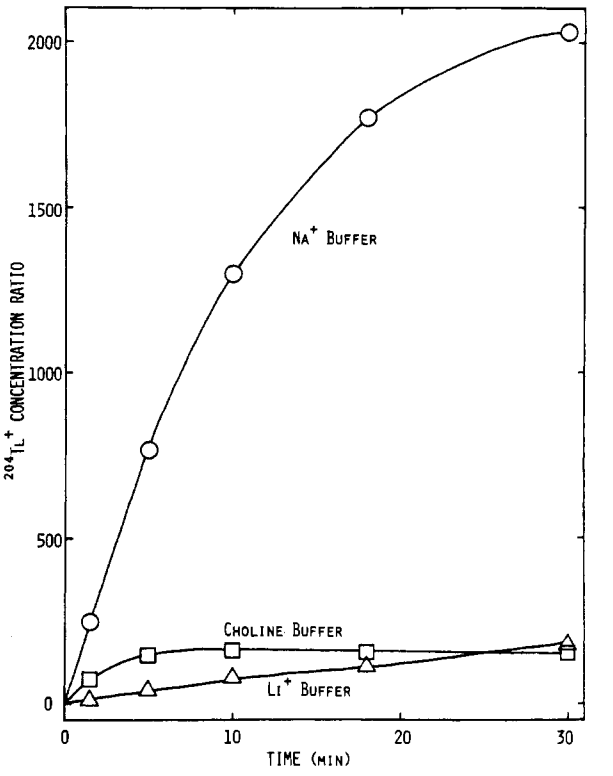

FIGURE 1: Effect of choline, Na^+, or Li^+ buffer on $^{204}Tl^+$ accumulation via the TrkA system. $^{204}Tl^+$ uptake was observed in TK1001 cells as described under Experimental Procedure. The assay buffers used were (○) Na^+, (△) Li^+, and (□) choline.

Rb^+ inhibits Tl^+ uptake via the TrkA system somewhat less effectively than K^+ (Figure 2). This is consistent with the observations of Rhoads et al. (1977) that the TrkA system discriminates against an $^{86}Rb^+$ tracer approximately 10-fold and supports the conclusion that the transport of Tl^+ via the TrkA system is similar to the transport of K^+ (Damper et al., 1979).

K^+ uptake is also stimulated by Na^+ and inhibited to some extent by Li^+ compared to choline (Figure 3). The accumulation ratios observed for K^+ (Figure 3) are somewhat higher than those for Tl^+ (Figure 1), perhaps due to inhibitory effects of high internal Tl^+ concentrations or to some discrimination of the TrkA system against Tl^+ compared to K^+.

Kdp System. Tl^+ accumulation via the Kdp system is also stimulated by incubation in an Na^+ buffer compared to a choline buffer (Figure 4). However, in contrast to its effect on the TrkA system, Li^+ stimulates Tl^+ uptake via the Kdp system better than Na^+ (Figure 4). This stimulation by Li^+ is probably due to the properties of the Kdp system rather than an artifact, because under similar conditions Tl^+ uptake via the TrkA system is inhibited (Figure 1).

The relative stimulatory effect of Na^+ and Li^+ on Tl^+ accumulation depends on the concentrations of Na^+ and Li^+ (Figure 5). Li^+ appears to stimulate Tl^+ uptake via the Kdp system better than Na^+ only when present in concentrations higher than ~15 mM with maximal stimulation occurring at ~80 mM Li^+. At concentrations lower than 15 mM, Na^+ enhances Tl^+ uptake better than Li^+. The maximal stimulation of Tl^+ uptake by Na^+, which is considerably less than the maximal stimulation by Li^+, occurs at ~10 mM Na^+.

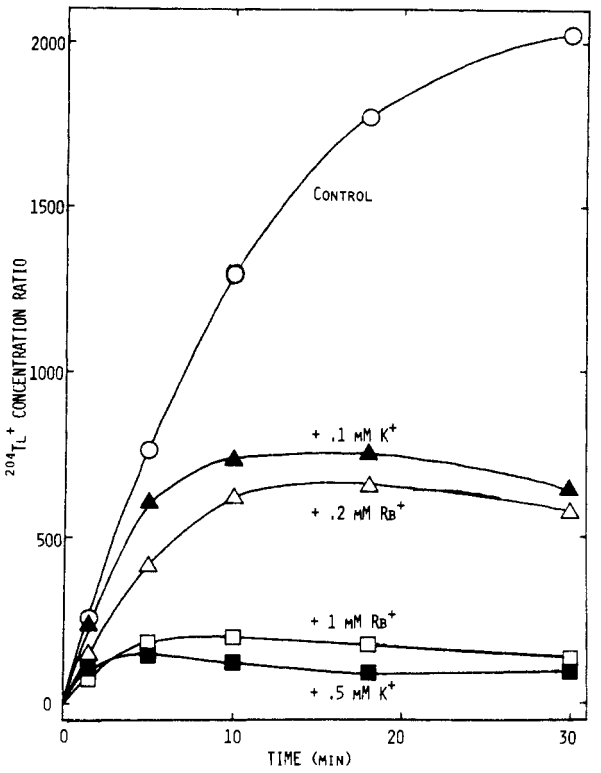

FIGURE 2: K^+ and Rb^+ inhibition of $^{204}Tl^+$ accumulation via the TrkA system. The assays were performed in Na^+ assay buffer with TK1001 cells as described under Experimental Procedure. Additions were (○) none, (▲) 0.1 mM KCl, (△) 0.2 mM RbCl, (■) 0.5 mM KCl, and (□) 1.0 mM RbCl.

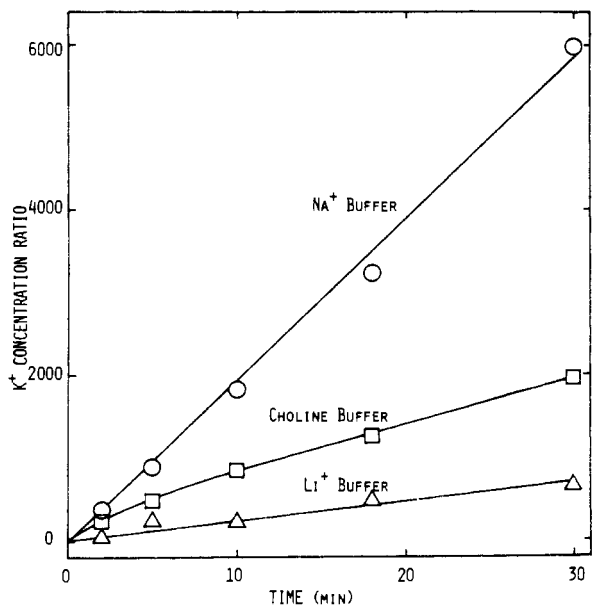

FIGURE 3: Effect of choline, Na^+, or Li^+ buffer on K^+ accumulation via the TrkA system. K^+ uptake was observed in TK1001 cells as described under Experimental Procedure. The assay buffers used were (○) Na^+, (△) Li^+, and (□) choline.

In addition, incubation in a Li^+ buffer enhances the effectiveness of Rb^+ as an inhibitor of Tl^+ uptake via the Kdp system. In an Na^+ buffer Rb^+ is a very poor inhibitor of Tl^+ uptake compared to K^+ (Figure 6). One millimolar Rb^+ (representing a 100-fold ratio of Rb^+ to Tl^+) only decreases Tl^+ accumulation ~40% whereas 0.1 mM K^+ (only a 10-fold ratio of K^+ to Tl^+) virtually abolishes Tl^+ uptake. K^+ of 0.02 mM probably inhibits quite effectively early in the experiment, but as most of the K^+ is rapidly accumulated, the apparent

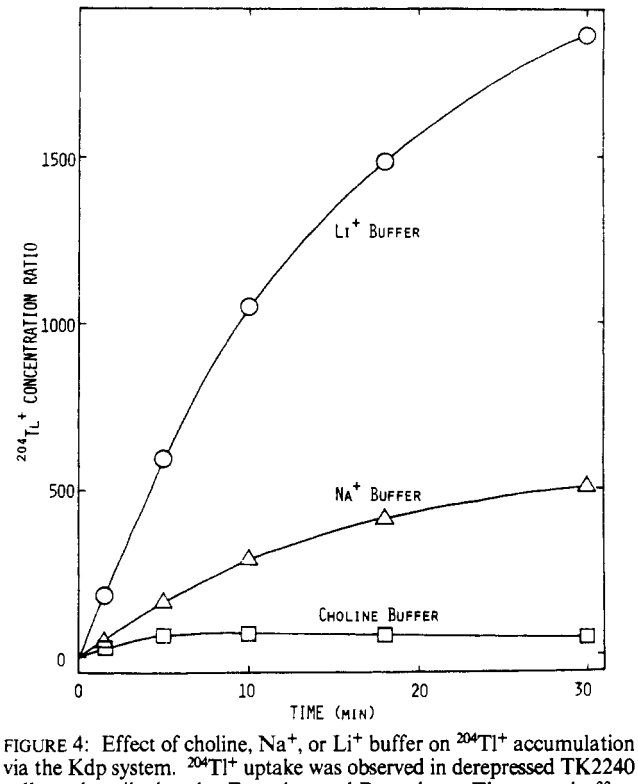

FIGURE 4: Effect of choline, Na^+, or Li^+ buffer on $^{204}Tl^+$ accumulation via the Kdp system. $^{204}Tl^+$ uptake was observed in derepressed TK2240 cells as described under Experimental Procedure. The assay buffers used were (○) Li^+, (△) Na^+, and (□) choline.

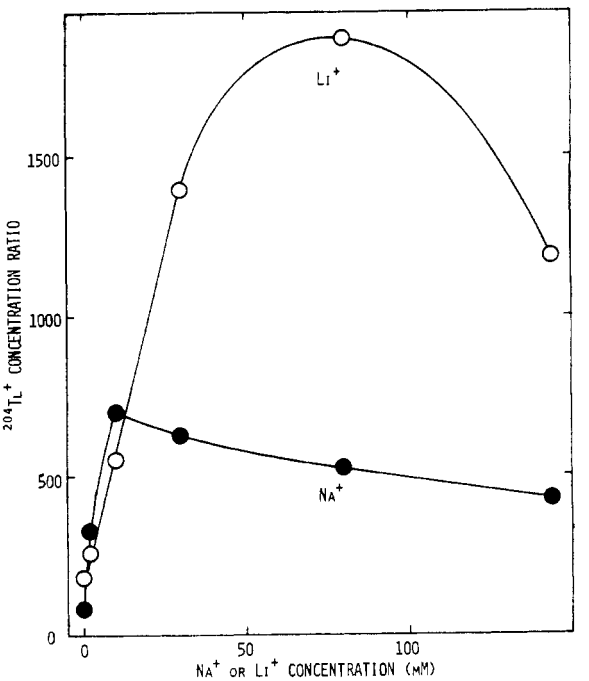

FIGURE 5: Stimulation of $^{204}Tl^+$ accumulation via the Kdp system as a function of the concentration of Na^+ or Li^+. A series of assays similar to those shown in Figure 4 were performed with derepressed TK2240 cells in which the concentration of Na^+ or Li^+ in the assay buffer was changed as described under Experimental Procedure. The $^{204}Tl^+$ concentration ratio obtained after 10 min in those assays is plotted here as a function of the concentration of Na^+ (●) or Li^+ (○).

inhibition decreases at later times. This is also consistent with the observation of Rhoads et al. (1977) that the Kdp system discriminates against an $^{86}Rb^+$ tracer approximately 1000-fold. The effectiveness of Rb^+ as an inhibitor of Tl^+ uptake via the Kdp system increases about 10-fold when a Li^+ buffer is used (Figure 7), although Rb^+ still does not inhibit as effectively

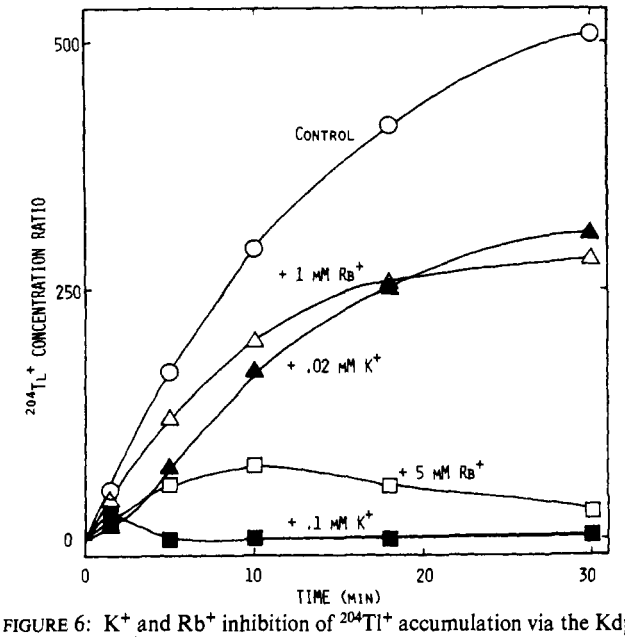

FIGURE 6: K^+ and Rb^+ inhibition of $^{204}Tl^+$ accumulation via the Kdp system in Na^+ assay buffer. Derepressed TK2240 cells were used as described under Experimental Procedure. Additions were (○) none, (▲) 0.02 mM KCl, (△) 1.0 mM RbCl, (■) 0.1 mM KCl, and (□) 0.5 mM RbCl.

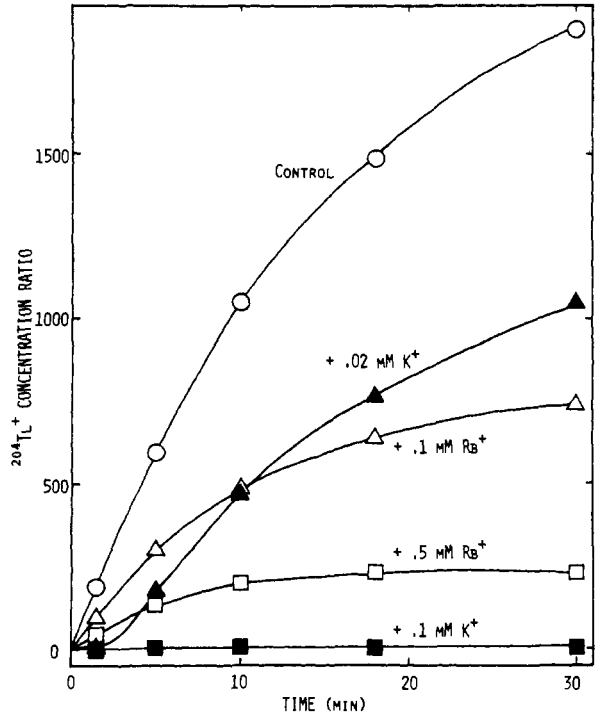

FIGURE 7: K^+ and Rb^+ inhibition of $^{204}Tl^+$ accumulation via the Kdp system in Li^+ assay buffer. Derepressed TK2240 cells were used as described under Experimental Procedure. Additions were (○) none, (▲) 0.02 mM KCl, (△) 0.1 mM RbCl, (■) 0.1 mM KCl, and (□) 0.5 mM RbCl.

as K^+. Li^+ does not appear to increase the effectiveness of K^+ as an inhibitor of Tl^+ uptake.

K^+ uptake via the Kdp system is also stimulated by Na^+ compared to choline (Figure 8). Li^+, while stimulating K^+ uptake somewhat compared to choline, is not nearly as effective as Na^+ (Figure 8). This is consistent with the observation that Li^+ does not appear to enhance the ability of K^+ to inhibit Tl^+ transport. The fairly high initial rate of K^+ uptake in all three buffers may be partly due to internal Na^+ accumulated in the

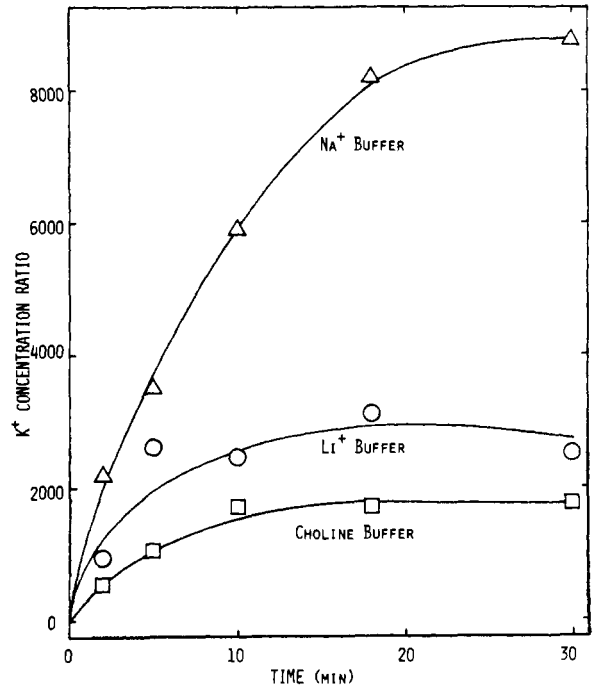

FIGURE 8: Effect of choline, Na^+, or Li^+ buffer on K^+ accumulation via the Kdp system. K^+ uptake was observed in derepressed TK2240 cells as described under Experimental Procedure. The assay buffers used were (○) Li^+, (△) Na^+, and (□) choline.

cells during the starvation process to deplete internal K^+ stores.

Discussion

The studies reported here show that in *E. coli* Na^+ stimulates the accumulation of both Tl^+ and K^+ via both the TrkA and Kdp transport systems. Na^+-stimulated $^{204}Tl^+$ uptake via a K^+-inhibitable system has recently been reported to occur in *Streptococcus lactis* (Kashket, 1979). Li^+ inhibits Tl^+ and K^+ uptake via the TrkA system but stimulates Tl^+ (and, to some extent, K^+) uptake via the Kdp system. Li^+ also appears to decrease the discrimination of the Kdp system against Rb^+. Several plausible models might account for these effects of Na^+ and Li^+ on Tl^+ and K^+ transport via the TrkA and Kdp systems.

Since the TrkA and the Kdp systems both require ATP for K^+ transport (Rhoads & Epstein, 1977), it is possible that they might function similar to the Na^+/K^+-ATPase of animal plasma membranes (Glynn & Karlish, 1975). The stimulation by Na^+ could then be due to Na^+ fulfilling the specificity requirements for countertransport of another cation better than Li^+ or choline. In both systems Na^+ provided the best stimulation when the uptake of K^+ was observed (Figures 3 and 8). This mechanism would provide a route for Na^+ extrusion from *E. coli* in addition to the Na^+/H^+ antiporter (West & Mitchell, 1974; Schuldiner & Fishkes, 1978; Beck & Rosen, 1979).

Since the TrkA system appears to require the presence of a protonmotive force in addition to ATP for activity (Rhoads & Epstein, 1977), a different explanation for the Na^+ stimulation of this system might be a mechanism where K^+ is cotransported with Na^+. The energy for transport could then be derived from the protonmotive force via the Na^+ electrochemical gradient established by the Na^+/H^+ antiporter. The driving force for K^+ uptake would then include the concentration gradient of Na^+ in addition to the membrane electrical potential. ATP might then be involved in the regulation of the system. This mechanism is essentially the converse of that postulated by Rhoads & Epstein (1977) for the TrkA system.

An analogous K^+/H^+ symporter has been proposed for *Streptococcus faecalis* (Bakker & Harold, 1980).

In either model the K^+ transporter would contain at least one binding site for K^+ and one for Na^+, presumably the normal substrates. In the presence of Na^+, Tl^+ accumulation closely parallels that observed for K^+ in that both the TrkA and the Kdp systems are stimulated and the observed discrimination against Rb^+ is similar to that observed by Rhoads et al. (1977) for K^+ transport. Li^+ inhibits both Tl^+ and K^+ accumulation via the TrkA system. Perhaps Li^+ is a very poor substrate for this system. Li^+, however, stimulates Tl^+ uptake via the Kdp system and is a much more effective stimulator than Na^+ at concentrations over 20 mM. This is not true for K^+ uptake. Li^+ also decreases the discrimination of the Kdp system for Rb^+. Possibly when Li^+, with a smaller crystal ionic radius than Na^+, interacts with the Na^+ site of the Kdp system, the effective size of the K^+ site is increased somewhat. This might decrease the interaction of the site with K^+ and facilitate the binding of the larger Tl^+ and Rb^+ ions. This could account for the large enhancement of Tl^+ uptake seen in the Li^+ assay buffer compared to the Na^+ assay buffer (Figure 4) in contrast to the decreased uptake of K^+ in the Li^+ assay buffer compared to the Na^+ assay buffer (Figure 8). In any case, although Li^+ and Tl^+ are probably not physiological substrates of the Kdp and TrkA K^+ transport systems, they are useful as probes for the interactions of Na^+ and K^+ with these systems.

In preliminary studies of the growth of TK2240 and T-K1001 on agar plates, we have been unable to observe a requirement for Na^+ for growth, although a requirement for K^+ is easy to observe. Thus, the cells can probably maintain internal levels of K^+ sufficiently high for growth even when very little Na^+ is present. Perhaps some other cation (H^+, NH_4^+, or choline) can serve well enough as a substitute for Na^+ to allow for growth.

The observations in this paper indicate that Na^+ is probably involved in the mechanism of K^+ accumulation via the Kdp and TrkA systems in *E. coli*. Future efforts will concentrate on determining what model best explains the effect of Na^+ on each K^+ transport system. At least one of these systems, the Kdp system, may be very similar to the Na^+/K^+-ATPase of higher organisms.

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Effect of Alkyl-Substituted Precursors of Cholesterol on Artificial and Natural Membranes and on the Viability of *Mycoplasma capricolum*†

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ABSTRACT: Various alkyl-substituted sterols and stanols representative of the intermediates in cholesterol biosynthesis from lanosterol have been compared with respect to (a) their effect on the physical state of lecithin vesicles, (b) their efficacy as growth factors for the sterol auxotroph *Mycoplasma capricolum*, and (c) their effect on the physical state of the respective mycoplasma membranes. By all three criteria, sterol effectiveness progresses in the order lanosterol < 4,4-dimethylcholestanol ≤ 4β-methylcholestanol < 4α-methylcholestanol < cholestanol < cholesterol. Since the corresponding steps in cholesterol biosynthesis occur in the same order, we conclude that the nuclear modifications of the lanosterol structure by oxidative demethylation serve to improve the membrane function of the sterol molecule.

The presence of sterols in membranes of all but the most primitive cells suggests that they perform an essential function in higher forms of life. At the physiological or biochemical level, this membrane function is not well understood. It can, however, be readily demonstrated that experimentally induced changes in cholesterol concentration alter the physical state of membranes and in turn modulate a broad range of cellular processes including lateral diffusion of receptors (Frye & Edidin, 1970), ion transport and solute permeability (Wiley & Cooper, 1975; Kimelberg & Papahadjopoulos, 1972), cell–cell interactions (Edelman, 1976), and the shape of cell surfaces (Heiniger et al., 1976).

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