Cation Transport in *Serratia marcescens* and *Serratia marnorubra*

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The sodium, potassium, and magnesium ion contents of *Serratia marcescens* and those of its salt-tolerant relative, *S. marnorubra*, were determined by atomic-absorption spectrometry. The intracellular K^+ and Mg^{2+} contents of both microorganisms were found to be dependent on the ionic strength of the growth or suspending medium. The Mg^{2+} content of *S. marnorubra* was generally greater than that of *S. marcescens*. The Na^+ content of the cells was normally low and did not increase as the cells aged or when the cells were grown in media of high ionic strength. The transport of K^+ by resting cells suspended in hypertonic solution was studied by chemical and light-scattering techniques and was found to be more rapid in *S. marcescens* than in *S. marnorubra*. The slower rate of K^+ transport in *S. marnorubra* is probably related to the lower glycogen reserves found in resting cells of this microorganism. K^+ transport was found to have a pH optimum of 5.5 to 6.1 for *S. marcescens*, and the K_m for K^+ was approximately 1.6 mM. Na^+ and Mg^{2+} were not taken up by the cells, although the presence of Mg^{2+} tended to decrease rates of K^+ uptake. Tris-(hydroxymethyl)aminomethane, routinely used for resuspending the cells, was apparently taken up by the cells at pH > 7.

Considerable research has been devoted in recent years to problems associated with biological ion transport; however, studies with bacterial systems have received relatively little attention. Nevertheless, ion transport across bacterial membranes is of particular interest because of the ability of these microorganisms to withstand wider variations in osmotic pressure than can any other organism. Their relative morphological simplicity makes bacterial cells extremely useful subjects for studies dealing with ion transport across biological membranes. The plasma membrane is the only osmotic barrier for small molecules entering or leaving the bacterial cells, and the enzymes and cofactors required for energy metabolism required for active transport are located either on or near the plasma membrane. They do not possess the endoplasmic reticula, mitochondria, nuclear membranes, and interstitial materials which make the interpretation of results obtained with higher organisms and tissues much more difficult.

The energy-dependent transport of organic and inorganic compounds has been described in a variety of bacteria. Active accumulation of these

compounds has been followed qualitatively by a number of workers by observing the changes in the amount of light scattered or transmitted by suspensions of plasmolyzed bacteria when the specific compounds were introduced into the suspending medium (1, 3, 4, 14, 18-20, 22, 28; A. Abrams, Fed. Proc. 17:178).

Other workers (5, 7, 9, 10, 15, 24-27, 29, 30) have used chemical techniques for determining both the internal concentrations and the rates of accumulation of potassium, sodium, and chloride ions in *Escherichia coli*, *Streptococcus faecalis*, *Alcaligenes faecalis*, *Aerobacter aerogenes*, and a number of halophilic bacteria. Those experiments measuring the uptake of radioactive isotopes of Na^+ and K^+ have been criticized (26) because of the inability of such techniques to distinguish between simple steady-state exchange processes and actual net uptake of the particular ion by the cells. Schultz and co-workers, working with Na^+ -rich, K^+ -poor stationary cells of *E. coli* (27) and *S. faecalis* (30), found that the resuspension of cells in fresh media caused rapid accumulation of K^+ and expulsion of Na^+ . The accumulation of K^+ in stationary *E. coli* cultures seemed to occur as the result of two parallel processes: (i) a rapid exchange of intracellular H^+ for K^+ in

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the suspending medium and (ii) a slower exchange of intracellular Na^+ for the extracellular K^+ . These exchange processes were affected by pH and were found to be strictly dependent on exogenous energy supplies, since both *E. coli* and *S. faecalis* under the experimental conditions had essentially no internal energy reserves. In later studies by the same workers (9, 10), the cellular K^+ content of exponential cells of *E. coli* was found to be determined primarily by the osmolarity of the growth medium, and sudden changes in osmolarity led to rapid changes in K^+ content. It was pointed out that the ability of *E. coli* to maintain internal osmotic pressure equal to or somewhat greater than that of the environment is important in assuring close contact between the membrane and cell wall, a relationship which is required for cell wall synthesis and cell division.

The studies reported in the present paper utilized a combination of light-scattering techniques and chemical analyses to determine and compare the intracellular concentrations of Na^+ , K^+ , and Mg^{2+} , and rates of K^+ accumulation in resting suspensions of *Serratia marcescens* and its salt-tolerant relative, *S. marinorubra*. The work was undertaken in the hope that comparisons of the behavior of these two closely related microorganisms (6) would reveal new information concerning membrane and physiological differences that may contribute to salt tolerance.

MATERIALS AND METHODS

Bacteria. The bacteria used in this study were *S. marcescens* HY and *S. marinorubra* S10 obtained from W. Belser, University of California at Riverside.

Media. The glycerol medium consisted of 20 g of glycerol, 1 g of Yeast Extract (Difco), and 2 g of Peptone (Difco) in 1,000 ml of distilled water. The final pH was adjusted to 6.8 to 7.0 by the addition of NaOH. The K^+ and Na^+ concentrations were approximately 0.95 and 2.44 mM, respectively.

Culture conditions. Cultures of 250-ml volume were grown in baffled 1-liter flasks (Bellco) with shaking at 30 C in either a reciprocal or gyratory shaker at 150 to 200 excursions per min. Growth was determined by measuring optical density of the culture at 660 nm by using a Beckman DB spectrophotometer or a Bausch and Lomb Spectronic 20 spectrophotometer.

Preparation of cells for light-scattering studies. Cells were harvested by centrifugation at 4 C, washed twice, and resuspended in the desired solutions. Clumps of cells were removed by filtration through a double thickness of Whatman no. 1 filter paper, and the suspension was adjusted to a final optical density of approximately 0.3 at 660 nm. Cell suspensions were maintained under aerobic conditions throughout the experimental procedures.

Light-scattering measurements. Light-scattering measurements were performed in a model 2000-D Brice-Phoenix Universal Light Scattering Photom-

eter, by using the 546-nm emission maximum of a mercury arc lamp with the photocell placed at 90° relative to the plane of incident light. Results were recorded on a Sargent model SRL recorder. Portions (3 ml) of appropriate cell suspensions in a Brice-Phoenix light-scattering cell (10 by 10 mm) were placed in the instrument. A base level of light scattering was recorded, and the desired additions were made with a microsyringe.

Preparation of samples for atomic absorption analysis. Original cultures (30 to 40 ml) were harvested at the desired phase of growth by centrifugation in polypropylene centrifuge tubes. The solutions used for washing and resuspending were varied to determine their effect on transport, retention, and recovery of intracellular salts. After washing to remove adhering salts that would interfere with the analysis, the cells were centrifuged, and the pellet was resuspended in 9 ml of either 0.05 M tris(hydroxymethyl)aminomethane (Tris; pH 6.8) or redistilled water. Concentrated HNO_3 (1 ml) was added to aid in disrupting the cells and to effect the release of intracellular cations. The suspensions were then heated in a boiling-water bath for 5 to 10 min, after which treatment the debris was removed by centrifugation, and the supernatant liquid was decanted into clean polystyrene tubes for subsequent analysis. Samples were analyzed for Na^+ , K^+ , and Mg^{2+} on a model 290 Perkin Elmer atomic-absorption spectrometer, and results were expressed as micromoles of the particular cation per 100 mg (dry weight) of cells, as estimated from the standard curve shown in Fig. 1. The standard curve was prepared by using cells of *S. marcescens* and *S. marinorubra* harvested in the exponential phase. The cells were washed twice in distilled water and desiccated in a vacuum at 50 C.

Glycogen determinations. Glycogen was determined on samples of cells washed twice in distilled water in a modification of a method described by Kerr (13). The cells were hydrolyzed in boiling 20% KOH. Ethanol was added to precipitate the glycogen, and the precipitate was washed once in absolute ethanol. The glycogen was hydrolyzed in 1 N HCl at 100 C and the resultant glucose was determined by the Glucostat method (Worthington Biochemical Corp.). Results were expressed in terms of milligrams of glycogen per gram of dry cells.

RESULTS

The transport of K^+ by resting cells of *S. marcescens* and *S. marinorubra* is an energy-dependent process that is disrupted by a number of common metabolic inhibitors. These organisms differ from *E. coli* and *S. faecalis* in that they do not require the addition of exogenous energy sources for the transport of K^+ . This is due to relatively large internal reserves of glycogen, particularly in *S. marcescens*.

The assimilation and retention of intracellular cations in these bacterial cells was found to be dependent on the age of the culture, the medium on which the cells were grown, and the type of

FIG. 1. Standard curve for estimating dry weights of cells. Data were taken with water-washed cells from exponential cultures of *S. marcescens* and *S. marinorubra*.

treatment before specific analytical procedures. It was found that best retention of intracellular constituents and least amount of variation occurred when the cells were washed in approximately isotonic solutions of $MgCl_2$. Control studies made on fresh and spent glycerol media indicated that 98 to 100% of the K^+ missing from spent media was recovered in the washed cells. In the case of media containing 3% NaCl, however, only 50% or less of the K^+ missing from the spent media remained with the cells during the washing procedure. For the determination of Mg^{2+} associated with the cells, no apparent differences were noted when the cells were washed with either distilled water or isotonic NaCl solutions.

The amounts of Na^+ , K^+ , and Mg^{2+} found in *S. marcescens* and *S. marinorubra* are shown in Table 1. The intracellular Na^+ content is very low under all conditions of growth of both microorganisms, although somewhat higher in exponential cells than in stationary cells. Considerable amounts of Na^+ were found, however, when cells of either organism were grown in 3% NaCl-glycerol and subsequently washed in distilled water. Such a condition was always associated with severe osmotic shock, considerable lysis, and displacement of intracellular K^+ by the Na^+

trapped in the intercellular spaces and, therefore, present in the washing solution.

The amount of intracellular K^+ was found to be directly related to the ionic strength of the growth medium. Growth of either organism in media containing 3% NaCl, KCl, or Na_2SO_4 resulted in similar increased intracellular concentrations of K^+ . In these salt-grown cells, much of the K^+ appears to be loosely associated with the cells, and consequently easily lost to wash solutions, even when these are of approximate isotonicity. Nevertheless, K^+ and small amounts of Mg^{2+} are primarily taken up under conditions of increased ionic strength of the growth media. An examination of spent 3% NaCl-glycerol media from cultures of *S. marinorubra* indicated that 4.33 mg of K^+ were missing from a volume of original culture required to yield 100 mg of cells. Assuming that the cells are similar in size and shape to *E. coli* and using the data of Schultz *et al.* (24), who found the dry-weight to wet-weight ratio to be 0.228 and the extracellular space was 0.20 ml/g (wet weight) of packed cells, the intracellular K^+ concentration was roughly estimated to be 0.44 molal. The concentration of this cation alone approximated the concentration of Na^+ in the external medium (0.51 molal), indicating that the cells experience no extreme differences in internal and external ionic strength during growth. The facility with which much of the internal K^+ of salt-grown cells is eluted by a variety of wash solutions suggests that a con-

TABLE 1. Intracellular cation content^a

Cation	<i>S. marcescens</i>		<i>S. marinorubra</i>	
	Exponential	Stationary	Exponential	Stationary
Glycerol-grown cells				
K^+	23.5	7.93	22.5	15.3
Na^+	1.61	0.217	0.696	0.087
Mg^{2+}	3.83	0.412	6.79	3.70
3% NaCl-glycerol-grown cells				
K^+	28.6	58.8	52.9	54.7
Na^+	1.48	0.304	0.870	0.304
Mg^{2+}	6.38	3.50	8.40	2.84

^a Values expressed as micromoles of cation per 100 mg (dry weight) of cells. For determinations of K^+ and Na^+ , cells were washed in 0.1 M or 0.5 M $MgCl_2$ for glycerol-grown or 3% NaCl-glycerol-grown cells, respectively, and treated as described in the text. For determination of Mg^{2+} , no significant differences were observed when the cells were washed in either isotonic NaCl or redistilled water.

siderable fraction is not bound to intracellular constituents. It is doubtful that such cation accumulation in media of high ionic strength can be attributed to ion-exchange properties of protoplasmic polyelectrolyte, as suggested for passive K^+ accumulation in *E. coli* by Damadian (8).

The amount of Mg^{2+} associated with cells of either organism decreased with age (Table 1). The Mg^{2+} content of *S. marinorubra* was consistently higher than that in comparable cells of *S. marcescens*, and the content in both organisms increased as the cells were taken from regular glycerol media to media containing 3% NaCl.

Effect of pH of washing solution on cation content. The effect of pH of the washing solution on retention of intracellular K^+ and Mg^{2+} by *S. marcescens* is shown in Table 2. As the pH was lowered to approximately 3, the cells lost most of their intracellular K^+ . At pH 2, the Mg^{2+} was effectively displaced by the acid. At these low pH values, the cells became extremely fragile and difficult to handle. The loss of K^+ was probably associated with the weakening of the chelate bonds between cell envelope and the Mg^{2+} by protonation of some of the acidic groups normally involved in chelation. Such displacement of Mg^{2+} would result in loss of membrane integrity.

Light-scattering studies. The use of light scattering to measure transport of materials across microbial membranes is based on the observation that some bacterial cells (particularly gram-negative forms) plasmolyze when they are exposed to a hypertonic environment (2, 4, 5, 11, 12, 16, 17, 21, 23). Plasmolysis is accompanied by an increase in the amount of light scattered by the cell suspensions.

The initial response of *S. marcescens* to increasing concentrations of NaCl is shown in Fig. 2. Figure 2 also shows a comparison between the

FIG. 2. Light-scattering response of *Serratia marcescens* to added NaCl. Exponential cells of *S. marcescens* were harvested from glycerol media and washed twice in redistilled water. Cells were resuspended in water (curve A) or 0.05 M Tris (pH 6.8; curve B) to a final optical density of 0.3. Increase in light scattered was measured after the addition of NaCl.

behavior of cells suspended in redistilled water and that of cells suspended in 0.05 M Tris buffer (pH 6.8). At pH 6.8, neither Na^+ nor Tris is rapidly taken into the cells; therefore, the suspending buffer (Fig. 2, curve B) contributes to the tonicity of the medium, and plasmolysis occurs at lower concentrations of added NaCl.

Cells of *S. marinorubra*, grown under similar conditions, respond to added salts in a fashion similar to that shown for *S. marcescens* (Fig. 2). Deplasmolysis of both organisms was dependent on the pH and on the type of salts or other transportable substances present in the suspensions and was accompanied by a decrease in the amount of light scattered by the cell suspensions. Deplasmolysis curves of both organisms when held in Tris-buffered 0.08 M NaCl or 0.08 M KCl are shown in Fig. 3. This concentration was chosen since it initially caused complete or nearly complete plasmolysis of these cell suspensions. K^+ was found to be transported rapidly into the cells at pH 6.8 (see Table 3), whereas Li^+ , Na^+ , and Tris were only very slowly transported, if at all. Under all conditions used in this study, no significant deplasmolysis was observed with the chloride salts of the divalent cations Mg^{2+} , Ca^{2+} , and Sr^{2+} . Cells of either organism grown in 3% NaCl-glycerol medium gave typical light-scattering curves if washed in isotonic $MgCl_2$. The concentration of salt required to give maximum

TABLE 2. Effect of pH on retention of intracellular K^+ and Mg^{2+} by glycerol-grown *S. marcescens*^a

pH of 0.85% NaCl wash	Cation ^b	
	K^+	Mg^{2+}
6.0	21.5	3.21
5.0	22.4	3.46
4.0	22.1	4.12
3.0	5.37	3.37
2.0	0.409	0.946

^a Exponential cells of *S. marcescens* were harvested and washed twice with 0.85% NaCl at the indicated pH; pH was adjusted by addition of HCl.

^b Values expressed as micromoles of cation per 100 mg (dry weight) of cells.

FIG. 3. Light-scattering changes produced by 0.08 M salt solutions with *Serratia marcescens* and *Serratia marinarubra*. Cells were grown in glycerol media to an optical density of approximately 0.2, washed twice in redistilled water, and resuspended in 0.05 M Tris (pH 6.8) to final optical density of 0.3. NaCl ($\blacktriangle$) or KCl ($\circ$) was added to a final concentration of 0.08 M to 3-ml portions of the resting cell suspension.

TABLE 3. Uptake of K^+ by water-washed cells of *S. marcescens* and *S. marinarubra*^a

Cells	Treatment	Cation ^b	
		Na ⁺	K ⁺
<i>S. marcescens</i>	Tris + 0.004 M KCl	0.348	25.2
	Tris + 0.02 M KCl	0.348	33.0
	Tris + 0.04 M KCl	0.304	34.8
	Tris + 0.06 M KCl	0.261	34.8
	Tris + 0.08 M KCl	0.174	26.8
	Tris + 0.16 M KCl	0.217	26.8
	Tris + 0.24 M KCl	0.261	26.8
	Tris + 0.32 M KCl	0.304	26.8
	Tris + 0.40 M KCl	0.217	27.6
	None	0.435	13.8
<i>S. marinarubra</i>	Tris	0.130	8.31
	Tris + 2.5 mM glucose	0.522	7.67
	Tris + 0.08 M KCl	0.565	19.9
	Tris + 0.08 M KCl + 2.5 mM glucose	0.304	23.1
	Tris + 0.16 M KCl	0.174	19.1
	Tris + 0.16 M KCl + 2.5 mM glucose	0.087	24.9
	Tris + 0.24 M KCl	0.130	20.3
	Tris + 0.24 M KCl + 2.5 mM glucose	0.130	23.7

^a Portions (30 to 40 ml) of glycerol-grown cells (exponential) were washed twice in water and treated for 10 min as indicated. Unless otherwise noted, Tris was at the usual concentration (0.05 M) at pH 6.8. Volume of suspension during treatment was 10 ml. After treatment, cells were washed twice with 0.1 M $MgCl_2$ and analyzed for Na⁺ and K⁺.

^b Values expressed as micromoles of cation per 100 mg (dry weight) of cells.

plasmolysis was, however, higher for these cells because of greater internal concentrations of K⁺ as described earlier. At higher pH values, up to pH 9, the rates of deplasmolysis with NaCl and Tris buffer became much greater. Atomic-absorption studies, however, failed to show any significant increase in the Na⁺ concentration of such cells (see Table 4). It is probable that deplasmolysis at pH >6.8 was largely due to the uptake of uncharged Tris molecules, which are more abundant at the elevated pH.

The addition of glucose to suspensions of either organism caused a marked increase in the rate of deplasmolysis and also in the amount of K⁺ accumulated by the cells during the course of the experiment, particularly in the case of *S. marinarubra*. This suggested that the lower rates of K⁺ accumulation and subsequent deplasmolysis in resting cells of *S. marinarubra* were an indication of its lower energy reserves. It is possible that some deplasmolysis may be attributed to the uptake of glucose, yet data such as that in Table 3

TABLE 4. Exclusion of Na⁺ by water-washed cells of *S. marcescens* and *S. marinarubra*^a

Cells	Treatment	Cation ^b	
		Na ⁺	K ⁺
<i>S. marcescens</i>	I		
	None	0.174	10.1
	Tris	0.522	11.3
	Tris + 0.08 M NaCl	0.391	11.3
	Tris + 0.16 M NaCl	0.609	10.3
	Tris + 0.24 M NaCl	1.09	9.59
	II		
	Tris (pH 6.8)	0.304	12.5
	Tris (pH 6.8 + 0.08 M NaCl)	1.17	11.7
	Tris (pH 7.5) + 0.08 M NaCl	2.22	11.4
<i>S. marinarubra</i>	Tris (pH 8.0) + 0.08 M NaCl	1.78	10.1
	Tris (pH 8.5) + 0.08 M NaCl	2.91	10.3
	Tris	0.435	5.55
	Tris + 2.5 mM glucose	0.217	5.12
	Tris + 0.08 M NaCl	2.57	6.68
	Tris + 2.5 mM glucose + 0.08 M NaCl	1.74	6.21
	Tris + 0.16 M NaCl	4.22	4.88
	Tris + 2.5 mM glucose + 0.16 M NaCl	3.52	6.90
	Tris + 0.24 M NaCl	4.22	4.88
	Tris + 2.5 mM glucose + 0.24 M NaCl	5.26	6.90

^a Conditions were as described in Table 3.

^b Values expressed as micromoles of cation per 100 mg (dry weight) of cells.

indicate that the amount of K^+ transported is also increased in the presence of glucose. Determination of intracellular glycogen content of resting cells of both organisms grown in glycerol media indicated significant differences in available energy reserves. Early exponential cells (optical density = 0.2) of *S. marcescens* and *S. marinorubra*, as used in most light-scattering experiments, contained 8.3 and 1.1 mg of glycogen, respectively, per gram (dry weight) of cells. Although no data are available for later stages of growth in *S. marinorubra*, the glycogen content of *S. marcescens* increased to approximately 30 mg/g (dry weight) as the culture approached the stationary phase of growth.

The uptake of K^+ (Table 3) was not accompanied by the expulsion of comparable amounts of Na^+ , which is in low concentration in these cells, nor of protons, as determined in separate experiments. Approximately 30 mg (dry weight) of water-washed *S. marcescens* grown in glycerol medium was resuspended in physiological saline or in redistilled water to a final optical density of 5.0 measured at 660 nm. No significant change in the pH of such resting cell suspensions was observed on the expanded-scale pH meter by the addition of plasmolyzing concentrations of KCl or NaCl, although K^+ was known to be taken up under such circumstances. Judging by the amount of cells present and the amount of K^+ known to be taken up, if H^+ were exchanged in a 1:1 ratio for K^+ , the pH of the suspension should have dropped over 3 pH units. The process of K^+ transport during suspension of *S. marcescens* in hypertonic salt solutions is obviously not the same as that proposed for stationary *E. coli* (9, 10, 24, 27).

Effect of pH on K^+ transport. The effect of altering pH on the rates of deplasmolysis in KCl is shown in Fig. 4. The data are a composite of rates shown by water-washed cells of *S. marcescens* suspended in 0.85% NaCl or 0.05 M Tris-hydrochloride, the pH of which was adjusted to the desired value by the addition of HCl. These solutions were chosen on the basis of their known effects on intracellular K^+ concentrations and light-scattering responses and to avoid the addition of unknown factors to the cell suspensions. Cells washed and resuspended in 0.1 M $MgCl_2$ gave rates that were approximately one-half those shown in the ordinate in Fig. 4; however, the same general curve was observed. There was an obvious pH optimum between pH 5.5 and 6.1.

Kinetic studies. The effect of various K^+ concentrations on the initial rates of deplasmolysis of glycerol-grown *S. marcescens* is shown in Fig.

FIG. 4. Effect of pH on rate of K^+ transport. Glycerol-grown *S. marcescens* (exponential) were washed twice with water, resuspended in 0.85% NaCl or 0.05 M Tris, and adjusted to the desired pH by the addition of HCl. Cells were plasmolyzed by the addition of 0.08 M KCl, and rates of deplasmolysis were observed.

5. The cells in this study were washed twice in 0.1 M $MgCl_2$ and resuspended in 0.05 M $NaHCO_3$ buffer at pH 5.5. At this pH which was found to be optimal for K^+ transport, the $NaHCO_3$ did not affect the rate of deplasmolysis. Before the addition of KCl to the cell suspensions, each 3-ml portion was plasmolyzed by the addition of $MgCl_2$ to a final concentration of 0.04 M. The

FIG. 5. Effects of various K^+ concentrations on rate of deplasmolysis of *S. marcescens*. Exponential cells of *S. marcescens* were washed twice in 0.1 M $MgCl_2$ and resuspended in 0.05 M sodium bicarbonate buffer (pH 5.5). Final optical density of the suspension was 0.3. Before the addition of KCl, 3-ml portions of cells were plasmolyzed by the addition of $MgCl_2$ to a final concentration of 0.04 M.

MgCl₂ concentration was held constant to insure that differences in rates of deplasmolysis were independent of concentration of this compound, as shown by results discussed above to retard rates of deplasmolysis. The initial rates of deplasmolysis were found to obey typical Michaelis-Menten kinetics, as determined by the Lineweaver-Burk plot. Half maximal velocity was obtained with 1.6×10^{-3} M KCl. These results were found to be reproducible when working with freshly prepared cells in NaHCO₃ buffer. Studies repeated in sodium phosphate buffer at pH 5.5 gave essentially the same rates and K_m value, without the pH and stability problems associated with the bicarbonate buffer. These kinetic data are in good agreement with those found for K⁺ transport in *E. coli* by Epstein and Schultz (9).

DISCUSSION

The transport of K⁺ by *S. marcescens* and *S. marinorubra*, unlike that reported for stationary cells of *E. coli*, is not accompanied by the simultaneous expulsion of Na⁺ or H⁺. These cells ordinarily do not contain large amounts of Na⁺. Exchange of Na⁺ for K⁺ and vice versa only appear when osmotically damaged cells and debris are resuspended in solutions of KCl or NaCl. It is doubtful, however, that this exchange process is dependent on metabolic energy. The transport of K⁺ that occurs when the cells are placed in hypertonic salt solutions is dependent on aerobic metabolism of endogenous glycogen reserves, glucose, or other utilizable growth substrate. The process is affected by pH and occurs optimally at pH 5.5 to 6.1. It has an apparent K_m of 1.6 mM KCl. Mg²⁺, though not actively taken up by the cells under these experimental conditions, has a marked influence on the rate at which K⁺ is transported.

The rate of K⁺ transport by *S. marinorubra* is considerably slower than that in *S. marcescens* and appears to be limited by the relative amounts of glycogen reserves within the cells. The addition of glucose to plasmolyzed cell suspensions enhanced the transport of K⁺ in both microorganisms. This fact suggested that the rate-limiting step in energy production required for cation transport is the conversion of glycogen to glucose.

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LITERATURE CITED

1. Abrams, A. 1960. Metabolically dependent penetration of oligosaccharides into bacterial cells and protoplasts. *J. Biol. Chem.* 235:1281-1285.
2. Avi-Dor, Y., M. Kuczynski, G. Schatzberg, and J. Mager. 1956. Turbidity changes in bacterial suspensions: kinetics and relation to metabolic state. *J. Gen. Microbiol.* 14:76-83.
3. Bovell, C. R., and L. Packer. 1963. Formate-controlled accumulation of inorganic and organic substances in *Escherichia coli*. *Biochem. Biophys. Res. Commun.* 13:435-438.
4. Bovell, C. R., L. Packer, and R. Helgerson. 1963. Permeability of *Escherichia coli* to organic compounds and inorganic salts measured by light scattering. *Biochim. Biophys. Acta* 75:257-266.
5. Brown, A. D. 1964. Aspects of bacterial response to the ionic environment. *Bacteriol. Rev.* 28:296-329.
6. Colwell, R. R., and M. Mandel. 1965. Adansonian analysis and deoxyribonucleic acid base composition of *Serratia marcescens*. *J. Bacteriol.* 89:454-461.
7. Cowie, D. B., R. B. Roberts, and I. Z. Roberts. 1949. Potassium metabolism in *Escherichia coli*. I. Permeability to sodium and potassium ions. *J. Cell. Comp. Physiol.* 34:243-257.
8. Damadian, R. 1969. Ion Exchange in *Escherichia coli*: potassium-binding proteins. *Science* 165:79-81.
9. Epstein, W., and S. G. Schultz. 1965. Cation transport in *Escherichia coli*. V. Regulation of cation content. *J. Gen. Physiol.* 49:221-234.
10. Epstein, W., and S. G. Schultz. 1966. Cation transport in *Escherichia coli*. VI. K exchange. *J. Gen. Physiol.* 49:469-481.
11. Henneman, D. H., and W. W. Umbreit. 1964. Factors which modify the effect of sodium and potassium on bacterial cell membranes. *J. Bacteriol.* 87:1266-1273.
12. Henneman, D. H., and W. W. Umbreit. 1964. Influence of the physical state of the bacterial cell membrane upon the rate of respiration. *J. Bacteriol.* 87:1274-1280.
13. Kerr, S. E. 1936. The carbohydrate metabolism of brain. I. The determination of glycogen in nerve tissue. *J. Biol. Chem.* 116:1-7.
14. Kleinzeller, A. G., and A. Kotyk (ed.). 1960. Membrane transport and metabolism. Academic Press Inc., New York.
15. Krebs, H. A., R. Whittam, and R. Hems. 1957. Potassium uptake by *Alcaligenes faecalis*. *Biochem. J.* 66:53-60.
16. Kuczynski-Halman, M., Y. Avi-Dor, and J. Mager. 1958. Turbidity changes in suspensions of gram-positive bacteria in relation to metabolic state. *J. Gen. Microbiol.* 18:364-368.
17. Mager, J., M. Kuczynski, G. Schatzberg, and Y. Avi-Dor. 1956. Turbidity changes in bacterial suspensions in relation to osmotic pressure. *J. Gen. Microbiol.* 14:69-75.
18. Mitchell, P., and J. Moyle. 1956. Osmotic function and structure in bacteria. *Symp. Soc. Gen. Microbiol.* 6:150-180.
19. Mitchell, P., and J. Moyle. 1956. Permeation mechanisms in bacterial membranes. *Disc. Faraday Soc.* 21:258-265.
20. Mitchell, P., and J. Moyle. 1959. Permeability of the envelopes of *Staphylococcus aureus* to some salts, amino acids, and non-electrolytes. *J. Gen. Microbiol.* 20:434-441.
21. Orskov, S. L. 1948. Experiments on active and passive permeability of *Bacillus coli communis*. *Acta Pathol. Microbiol. Scand.* 25:277-283.
22. Packer, L., and M. Perry. 1961. Energy-linked light scattering changes in *Escherichia coli*. *Arch. Biochem. Biophys.* 95:379-388.
23. Rogers, D., and S. Yu. 1963. Turbidity change during glucose permeation in *Escherichia coli*. *J. Bacteriol.* 85:1141-1149.
24. Schultz, S. G., and A. K. Solomon. 1961. Cation transport in *Escherichia coli*. I. Intracellular Na and K concentrations and net cation movement. *J. Gen. Physiol.* 45:355-369.
25. Schultz, S. G., N. L. Wilson, and W. Epstein. 1962. Cation

- transport in *Escherichia coli*. II. Intracellular chloride concentration. *J. Gen. Physiol.* 46:159-166.
26. Schultz, S. G., W. Epstein, and D. A. Goldstein. 1962. Cation transport in *Escherichia coli*. III. Potassium fluxes in the steady state. *J. Gen. Physiol.* 46:343-353.
27. Schultz, S. G., W. Epstein, and A. K. Solomon. 1963. Cation transport in *Escherichia coli*. IV. Kinetics of net K uptake. *J. Gen. Physiol.* 47:329-346.
28. Sistrom, W. R. 1958. On the physical state of the intracellularly accumulated substrates of beta-galactoside-permease in *Escherichia coli*. *Biochim. Biophys. Acta* 29:579-587.
29. Tempest, D. W., J. W. Dicks, and J. R. Hunter. 1966. The interrelationship between potassium, magnesium and phosphorus in potassium-limited chemostat cultures of *Aerobacter aerogenes*. *J. Gen. Microbiol.* 45:135-146.
30. Zarlengo, M. H., and S. G. Schultz. 1966. Cation transport and metabolism in *Streptococcus fecalis*. *Biochim. Biophys. Acta* 126:308-320.