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TECHNICAL MANUSCRIPT 482

PERMEABILITY OF SERRATIA MARCESCENS TO SOME INORGANIC SALTS

Leonard Zimmerman

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TECHNICAL MANUSCRIPT 482

PERMEABILITY OF *SERRATIA MARCESCENS* TO SOME INORGANIC SALTS

Leonard Zimmerman

Physical Science Division
BIOLOGICAL SCIENCES LABORATORIES

Project 1B522301A080

October 1968

ABSTRACT

The physical interactions between Serratia marcescens and solutions of NaCl, $CaCl_2$, CaI_2, NaI, and (Na_2HPO_4 + NaH_2PO_4) were examined. Dilute (0.017 N) salt solutions did not cause cells to lose water, as evidenced by the unchanged weight of centrifugally packed cells. The cells preferentially adsorbed the cations and repelled the anions of most salts in these solutions. Concentrated (1.71 N) salt solutions markedly reduced the weight and water content of centrifugally packed cells, although these took up considerable amounts of salts. More than 90% of the water in the packed-cell pellets was available for the solution of NaCl at 4.2 to 4.4% concentration. The observation that salts apparently penetrated the entire cells freely and yet caused extensive dehydration was not readily compatible with conventional concepts of solute-induced plasmolysis. Alternative hypotheses to explain the data included the following: (i) The cells lost weight and water to concentrated salt solutions through a non-osmotic competitive dehydration, causing a shrinkage of the protoplasmic gel. The shrinkage of the cell wall was limited because of the rigidity of its mucopeptide layer; therefore, a space appeared between the cell wall and the cell membrane. (ii) Cells may have equilibrated their water activity with that of their environment by two mechanisms: (a) the loss of water by plasmolysis or competitive dehydration, (b) alterations in cell permeability that admitted previously excluded solutes to the cell interior. Possibly the true explanation of the observations reported here will involve elements of all three hypotheses: plasmolysis, competitive dehydration, and permeability alterations.

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I. INTRODUCTION

Many gram-negative bacteria, including Serratia marcescens and Escherichia coli, survive quite well in the stationary phase of growth in suspensions containing little or no added solutes. Addition of NaCl or other inorganic salts is often followed by a stable increase in the optical density of such cell suspensions and, if the salt concentration is made 0.05 M or greater, separation of the cell wall from the cell membrane may be observed microscopically.[1-6] This evidence of plasmolysis, the reduction in cell volume accompanying the osmotic loss of water, occurs only among gram-negative organisms; plasmolysis of gram-positive cells results in osmotic shrinkage of the organism as a whole.[7] It is generally accepted that the cell membranes of plasmolyzed microorganisms are impermeable to NaCl, while the cell walls are permeable; if this were not the case, then the salt solution would not maintain plasmolysis.[8] One recent reviewer, however, has remained unconvinced by the experimental evidence supporting these concepts.[9]

On the other hand, NaCl (and some other salts) protected freeze-dried E. coli against the antibacterial effects of oxygen.[10,11] Alterations in NaCl concentration of S. marcescens suspensions were followed by marked changes in ability of these cells to survive freeze-thawing or freeze-drying.[12] These last observations raised some question as to whether the observed effects of the salts upon cell viability were due only to their extracellular presence. It seemed that the solutes in solutions that plasmolyzed the cells must be nonpenetrative, but literature that provided direct analytical verification of this hypothesis could not be found. Therefore, experiments were designed to determine the distribution of NaCl between the intra- and extracellular space in suspensions of S. marcescens containing plasmolytic and nonplasmolytic salt concentrations. The results of this investigation are not in complete agreement with those predicted by currently accepted concepts.

II. MATERIALS AND METHODS

As described elsewhere, the cells used in these studies were grown in a glucose-tryptose medium and then centrifuged, resuspended in mother liquor, and frozen into pellets.[13] Quantities of frozen pellets were thawed, and weighed amounts were dispensed into centrifuge tubes to permit the preparation of packed-cell pellets. Two milliliters of thawed pellet slurry contained about 2×10^{12} viable S. marcescens and yielded a wet packed-cell pellet weighing about 1.0 g. This population-per-gram was characteristic of pellets containing >90% viable S. marcescens.[14] Thus these studies evaluated the response of viable cells, not dead residues, to changes in their ionic environment.

In a typical experiment, four replicate packed-cell pellets of S. marcescens, each weighing about 5.0 g, were prepared by centrifugation at 20,000 x g for 10 minutes; these cells had been washed previously four times with distilled water. Each of the packed-cell pellets was resuspended in 3.0 ml of either 0, 0.1, 1, or 10% NaCl. The resulting suspensions were held at room temperature for 15 minutes and again centrifuged at 20,000 x g for 10 minutes.* These procedures were modeled after those described by MacDonald and Gerhardt[15] and by Conway and Downey.[16] The resulting supernatant liquid was decanted from the packed-cell pellet, and the volume, density, and Na^+ and Cl^- concentrations of the fluid were determined analytically. The total amount of Na^+ and Cl^- in the supernatant fluid could be calculated from these values. The difference between the amount of each ion found in the supernatant and the total amount of ion initially added revealed the amounts of cation and anion that remained associated with the packed-cell pellet. The results obtained with this material-balance technique were unexpected and, therefore, the data calculation procedures will be described in detail together with the results generated by their use.

Na^+ and Ca^{++} concentrations were determined by flame spectrophotometry: halide anion concentrations were estimated spectrophotometrically from the color developed in samples upon addition of mercuric thiocyanate followed by ferric ammonium sulfate.[17] Phosphate was determined by the method of Chen, Toribara, and Warner.[18] Urea concentrations were determined by addition of Nessler's reagent to samples that had been treated with urease to liberate ammonia. Dextrans have been used, as nonpenetrating solutes, to define the extracellular volume in wet packed-cell pellets.[6] The effects of various NaCl concentrations on this extracellular space were determined with Blue Dextran 2000;** dextran concentrations were estimated spectrophotometrically at 650 mμ. Dry weights of cell pellets were obtained by holding them under <1 μ Hg pressure over Drierite at room temperature until they reached constant weight, a process that required from 5 to 15 days.

All data recorded here are the means of not less than three replicate assays. Run-to-run variability in the mean weight of water-washed wet packed cells was about ±100 mg, but the weight changes following resuspension and recentrifugation were reproducibly characteristic for each suspending fluid.

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* Exploratory trials indicated that suspensions created in NaCl solutions yielded the same patterns of solute and solvent distribution whether centrifuged after 15 minutes at room temperature or after 18 hours' storage at 2 C.

** Pharmacia, Inc., Uppsala, Sweden.

III. RESULTS

A. EFFECTS OF NaCl ON SPACE AVAILABLE FOR DILUTION OF DEXTRAN IN PACKED-CELL PELLETS

Water-washed packed-cell pellets of S. marcescens were resuspended in 0.1% solutions of Blue Dextran 2000 (1 mg/ml) containing either 10, 1, 0.1, or 0% NaCl and recentrifuged. The dextran concentration of the supernatant fluid was determined, and the weight of the residual packed-cell pellet was determined gravimetrically, yielding the data in Table 1. Both the weight of the packed-cell pellets and the dextran concentration (mg/ml) in the supernatant fluids decreased with increasing salt concentrations. This decrease in the dextran concentrations indicated that the salt induced a progressive translocation of water from the intracellular to the extracellular spaces in the cell suspension.

TABLE 1. SUPERNATANT CONCENTRATIONS OF BLUE DEXTRAN AND WEIGHT OF PACKED-CELL PELLETS OBTAINED BY CENTRIFUGATION OF SALT-CONTAINING SUSPENSIONS OF S. MARCESCENS

NaCl Concentration of Added Solution[a]		Weight of Packed-Cell Pellet, g[a]		Dextran Concentration in Supernatant, % of Original
Normality	%	Before Resuspension in NaCl Solution	After Centrifugation from NaCl Solution	
0	0	4.738	4.713	82.9
0.0171	0.1	4.733	4.652	77.1
0.171	1.0	4.741	4.188	67.3
1.71	10.0	4.736	4 151	68.0

a. Packed-cell pellet created by centrifugation for 10 minutes at 20,000 x g, resuspended in 3.0 ml of indicated salt solution containing 0.1% Blue Dextran, held 15 minutes, then recentrifuged for 10 minutes at 20,000 x g.

B. Na^+ AND Cl^- RETENTION BY PACKED-CELL PELLETS

The preceding experiments showed that valid estimates of salt uptake or retention by packed-cell pellets could not always be obtained using methods that assumed that the volumes of cells and suspending fluids remained unchanged after mixing and recentrifugation. Attempts to make direct measurements of the volume changes of the cell pellets and suspending fluids were unsuccessful. Therefore, the uptake of Na^+ and Cl^- by packed-cell pellets of S. marcescens was investigated by means of the material-balance calculations outlined under Section II. The amounts and percentages of ions taken up by S. marcescens from NaCl solutions of varied strength are shown in Tables 2 and 3, together with the detailed sequences of operations and calculations. The resulting data indicated that:

1) Resuspension of cells in 0.1% NaCl did not materially decrease the weight of the recentrifuged pellet, which retained about 25% of the added Cl^- and about 50% of the added Na^+. Because theory required that electrical neutrality in the salt solution be maintained, it was assumed that the excess Na^+ uptake was due to exchange-adsorption, whereby the negatively charged water-washed cell surfaces took up about 0.75 mg of Na^+ in exchange for H^+. Qualitatively consistent with this assumption was the fact that the supernatant fluids became progressively more acid as more and more salt was added to the cells, changing from pH 7 in the absence of salt to pH 5.8 in the presence of added 10% NaCl.

2) Resuspension of cells in 1% NaCl markedly reduced the weight and the water content of the recentrifuged cell pellet. Nevertheless, from 33 to 40% of the added salt remained associated with the packed-cell pellet despite the reduced water content of the latter. Again the cells took up more Na^+ than Cl^- but the difference in uptake of these two ions, again ascribed to Na^+ exchange-adsorption, was only 2.2 mg. One possible explanation of the salt uptake by the water-depleted packed-cell pellet was that the salt (Na^+ and Cl^-) had entered the cells. The concept that a salt in solution could both penetrate and dehydrate cells was difficult to accept but equally as difficult to avoid.

3) Cells resuspended in 10% NaCl showed a greater reduction in weight and volume of the packed-cell pellet than cells resuspended in 1% NaCl, probably because the cells had to lose additional water so that their internal water activity would come into equilibrium with that of the extracellular solution. If the suspending fluid were inducing osmotic shrinkage or plasmolysis because the salt it contained could not enter the cells, then most of the salt should have been demonstrable in the supernatant fluid; but almost half of the salt remained with the packed-cell pellet. Na^+ and Cl^- were taken up by these cells in equal quantities, so that a differential adsorption of cations versus anions was not detectable. Assuming that the NaCl in the packed-cell pellet (141 mg) was present at no greater concentration than the NaCl in the suspending fluid (42 mg/ml),

then the cellular NaCl would have been distributed in at least 3.36 ml of solution. The weight of the packed-cell pellet was 4.078 g and, if 80% of the cell-pack weight were water, the cell pellet then would contain about 3.27 ml H_2O. The agreement between these numbers (3.36 ml versus 3.27 ml) suggested that all of the water in the packed-cell pellet might be available for the solution of NaCl. Therefore it appeared that the NaCl was entering the cells themselves, even though concentrated salt solutions were inducing the loss of weight and water from the cell pellet.

Direct evaluation of this hypothesis was performed as follows: Cell pellets were created by centrifuging twice-water-washed *S. marcescens* at 20,000 x g for 10 minutes. The pellets were weighed, resuspended in 3 ml of water or 10% NaCl solution, then centrifuged at 20,000 x g for 120 minutes to squeeze as much water as possible out of the cell pellet and to minimize the extracellular space in the pellet available for the solution of NaCl. Evaporative water loss during centrifugation was prevented by use of centrifuge tube caps. The supernatant fluid was then separated from the resulting cell pellet, and the assay procedures outlined in Table 4 were performed. The final volume of the supernatant fluid was considerably larger than the 3.0 ml initially added and also larger than the volume created by centrifugation for 10 minutes (Table 2, Column F). The supernatant fluid of the pellet treated with NaCl contained about 205 mg NaCl, leaving about 95 mg of salt in the cell pellet. The water contents of the cell pellets were also determined analytically. If the 95 mg of NaCl in the packed-cell pellets were in solution at the same concentration as the NaCl in the supernatant fluid, 44.2 mg/ml, then the resulting salt solution would have had a volume of 2.167 ml. This calculated salt solution volume was 94.9% of the actual water content of the cell pellet. These data indicated that all of the water in the packed-cell pellet, both intracellular and extracellular, was available for the solution of NaCl at 4.4% concentration.

This inference was tested by resuspending cell pellets in 3 ml and in 20 ml of 10% NaCl or water and recentrifuging them for 120 minutes. As shown in Table 5, cell pellets sedimented from 3 ml or 20 ml of water had equal weights. Pellets sedimented from 20 ml of salt solution took up more salt (193 mg) and left a more concentrated suspending fluid (8.1% NaCl) than the pellets centrifuged out of 3 ml of salt solution (100 mg and 4.3% NaCl). The net or salt-free weights of these pellets were equivalent, indicating that (i) an 8.1% NaCl solution did not withdraw more water from cell pellets than a 4.3% NaCl solution, and, therefore, (ii) cell pellets were probably dehydrated as completely as possible under these conditions by 4.3% NaCl solution.

TABLE 2. CELL-PACK DATA OBTAINED BY CENTRIFUGATION OF SALT-CONTAINING SUSPENSIONS OF S. MARCESCENS

Wet Cell-Pack Weight, g A	Solution Added, 3.0 ml B	A + B, g C	Recentrifuged Packed Cells, g D	C - D = Supernatant, g E[a]	Volume of E, Supernatant, ml F	Excess Volume, F - B G	% Weight Loss of Cells, 100 x G/A
4.782	H_2O	7.777	4.726	3.038	3.038	0.038	0.8
4.775	0.1% NaCl	7.776	4.703	3.050	3.050	0.050	1.0
4.770	1.0% NaCl	7.786	4.115	3.660	3.660	0.660	13.8
4.777	10% NaCl	7.990	4.087	3.888	3.782[b]	0.829[c]	17.4

a. Column C represents the sum of the values in columns A and B before mixing. A small amount of cell suspension was always lost on the stirrers used to disperse the cells in the salt solution. This quantity, ordinarily 0.020±0.010 g, was neglected in the calculations and represents the difference between the values entered in column E and those obtained by subtracting column D values from those in column C.

b. Weight of solution E divided by its density.

c. Corrected for apparent volume of salt in solutions B and F. This correction was insignificant for the less concentrated salt solutions.

TABLE 3. DISTRIBUTION OF Na^+ AND Cl^- BETWEEN PACKED-CELL PELLETS AND SUPERNATANT FLUIDS IN CENTRIFUGED SALT-CONTAINING SUSPENSIONS OF *S. MARCESCENS*

Solution Added, 3.0 ml	NaCl Added, mg	Supernatant Values					Packed Cell Values			
			Na^+ a/		Cl^- a/		Na^+		Cl^-	
		Volume	mg/ml	Total	mg/ml	Total	mg b/	% c/	mg b/	% c/
Water	0	3.038	0.09	0.27	0.03	0.09	-	-	-	-
0.1% NaCl	3.0	3.050	0.47	1.43	0.72	2.20	1.57	52.3	0.80	26.7
1% NaCl	30.0	3.660	4.9	17.9	5.5	20.1	12.1	40.3	9.9	33.0
10% NaCl	300.0	3.782	42	158.8	42	158.8	141.2	47.1	141.2	47.1

a. Expressed as the amount of NaCl that would contain the measured amount of Na^+ or Cl^-.
b. Values obtained by difference between amounts of Na^+ and Cl^- added and amount found in supernatant.
c. Percentages of added Na^+ and Cl^- missing from supernatant.

TABLE 4. ANALYSIS OF THE DISTRIBUTION OF SALT AND WATER IN CENTRIFUGED SUSPENSIONS OF S. MARCESCENS

Line	Treatment and Calculation Sequence	Suspending Fluid Water	Suspending Fluid 10% NaCl
1	Supernatant fluid[a]		
	a. Volume, ml	3.716	4.621
	b. NaCl concentration, mg/ml	-[b]	44.2
	c. Total NaCl content, mg	-	205.2
2	Cell pellet[a]		
	a. Water content, g and ml	3.187	2.283
	b. NaCl content, mg	-	95.8
	c. Volume (ml) required to keep NaCl in (2,b) in solution at concentration in supernatant (1,b)	-	2.167
3	Calculated percentage of water in cell pellet available for solution of NaCl[c]	-	94.9

a. Obtained by centrifuging S. marcescens for 10 minutes at 20,000 x g, resuspending in 3.0 ml of indicated fluid, then recentrifuging at 20,000 x g for 120 minutes.

b. Not measured.

c. $100 \times \frac{\text{calculated volume in line 2,c}}{\text{measured volume in line 2,a}}$

TABLE 5. WET WEIGHTS OF PACKED *S. MARCESCENS* PELLETS CENTRIFUGED FROM SUSPENSIONS OF VARIOUS VOLUME AND SALT CONCENTRATION

Resuspending Fluid	Concentration of NaCl in Final Supernatant, %	NaCl in Cell Pack, mg	Cell-Pack Wet Weight, g		
			Initial[a]	Final[b]	Net[c]
3 ml H_2O	-[d]	-	4.997	4.242	4.242
20 ml H_2O	-	-	4.991	4.249	4.249
3 ml 10% NaCl	4.3	100	4.998	3.468	3.368
20 ml 10% NaCl	8.1	193	4.993	3.574	3.381

a. Pellets centrifuged out of water suspension for 10 minutes at 20,000 x g.
b. Initial pellets resuspended in indicated fluid and recentrifuged for 120 minutes at 20,000 x g.
c. Final cell pack weight less weight of NaCl in the pellet.
d. Not measured.

Urea has been reported to be freely penetrative into S. marcescens.[5] If NaCl in concentrated solutions were also freely penetrative, then packed-cell pellets centrifuged from solutions containing NaCl and urea should retain equivalent percentages of each solute. This inference was tested experimentally. As shown in Table 6, the amount of urea retained by cell pellets was markedly reduced by the presence of 10% NaCl (Treatment 2 versus Treatment 4) probably because of the dehydrating effects of the salt. Nevertheless, urea and NaCl yielded similar patterns of distribution when their mixed solution was used as a resuspending fluid (Treatment 4); about 40% of each solute was retained in the packed-cell pellets and 60% remained in the supernatant fluid. These data indicate that S. marcescens was equally permeable to urea and to NaCl in concentrated solution.

TABLE 6. RETENTION OF ADDED UREA AND NaCl IN PACKED-CELL PELLETS OF S. MARCESCENS[a]

Treatment No.	Solute Concentration and Weight	Urea		NaCl	
		mg[b]	%[c]	mg[b]	%[c]
1	H_2O (none)	0	0	0	0
2	1% urea (30 mg)	16.5	55	0	0
3	10% NaCl (300 mg)	0	0	125	42
4	10% NaCl (300 mg) + 1% urea (30 mg)	12.0	40	125	42

a. Cells in water packed centrifugally at 20,000 x g for 10 minutes, resuspended in 3 ml of indicated solution, and recentrifuged at 20,000 x g for 10 minutes.
b. Quantity of added solute retained in packed-cell pellets.
c. Percentage of added solute retained in packed-cell pellets.

C. EFFECTS OF OTHER SALTS

The responses of S. marcescens to solutions of other salts were evaluated by means of the techniques used with NaCl solutions, e.g., washing the cells four times with water and centrifuging for 10 minutes to obtain packed-cell pellets. The salts tested were NaCl, NaI, CaI_2, $CaCl_2$, and a mixture of Na_2HPO_4 and NaH_2PO_4 (henceforth called $Na_xH_xPO_4$).* These were used in solutions whose concentrations differed by a factor of ten, each one being

* Prepared as a mixture containing 5.665% Na_2HPO_4 and 2.360% $NaH_2PO_4 \cdot H_2O$, the 1.71 N solution; this had a pH of 7.0.

equinormal with 0.1%, 1% or 10% NaCl (which is 1.71 N). The effects of these salt solutions on the water content of the packed-cell pellets were uniformly similar to those of NaCl (Table 7). The most dilute solutions had negligible effects, while the stronger salt solutions caused progressively greater losses in water content. The amounts of cations and anions from these salt solutions that were retained in the packed-cell pellets were also determined (Table 8).

TABLE 7. DEHYDRATION OF S. MARCESCENS BY SALT SOLUTIONS[a]

Salt	Percentage Loss of Water from Cell Pellets Suspended in and Centrifuged from Indicated Salt Solutions			
	0.0	0.0171 N	0.171 N	1.71 N
NaCl	1.0[b]	1.9	13.5	18.9
NaI	1.0	1.5	10.3	18.0
$CaCl_2$	1.0	0.4	8.3	11.3
CaI_2	1.0	1.9	9.0	14.9
$NaxHxPO_4$[c]	1.0	1.4	7.3	14.1

a. Cell pellets packed centrifugally at 20,000 x g for 10 minutes, resuspended in 3.0 ml of indicated fluid, and recentrifuged at 20,000 x g for 10 minutes.

b. Grand mean of all control treatments.

c. 1.71 N sodium phosphate solution contained

5.665% Na_2HPO_4, which is 0.399 M or 1.197 N
and 2.360% $NaH_2PO_4 \cdot H_2O$, which is 0.171 M or 0.513 N
Total normality=1.710 N

TABLE 8. CATION AND ANION UPTAKE BY S. MARCESCENS CELL PELLETS[a]

	Percentages of Salt Ions Remaining in Cell Pellets Suspended in and Centrifuged from Indicated Salt Solutions					
	0.017 N		0.17 N		1.71 N	
Salt	Cation	Anion	Cation	Anion	Cation	Anion
NaCl	53	33	40	39	45	44
NaI	44	45	43	39	45	44
$CaCl_2$	94	50	50	39	49	47
CaI_2	88	48	43	42	53	44
$Na_xH_xPO_4$[b]	51	15	46	26	41	39

a. Packed centrifugally at 20,000 x g for 10 minutes, resuspended in 3.0 ml of indicated fluid, and recentrifuged at 20,000 x g for 10 minutes.

b. 1.71 N sodium phosphate contained
5.665% Na_2HPO_4, which is 0.399 M or 1.197 N
and 2.360% $NaH_2PO_4 \cdot H_2O$, which is 0.171 M or 0.513 N
Total normality = 1.710 N

Nonuniform retention of cations and anions from 0.0171 N salt solutions was observed as follows: about 50% of the cation and 30% of the anion was retained from NaCl; about 90% of the cation and 50% of the anion was retained from $CaCl_2$ and CaI_2; and about 50% of the cation and 15% of the anion was retained from $Na_xH_xPO_4$. These effects were ascribed to a combination of cation adsorption and anion repulsion by the negatively charged cell surfaces, with the multivalent ions responding more strongly than the monovalent ones. On the other hand, NaI behaved like an un-ionized solute in that its cations and anions were taken up in equal amounts at this and at the higher concentrations.

These varied patterns of cation and anion uptake were not evident when cells were exposed to more concentrated salt solutions. Apparently $Na_xH_xPO_4$ was taken up less than were the other salts, possibly because its highly charged anion was so strongly repelled by the cells. The relatively uniform salt uptake from the concentrated solutions of the other salts was taken as evidence that the diverse ion retention patterns observed among the dilute salt solutions were probably due to cation adsorption and anion repulsion. By analogy with the evaluation of the distribution of NaCl, it was inferred that all of these salts, in solutions of sufficiently high concentration, were able both to dehydrate and to penetrate the cells, thereby creating equal intracellular and extracellular concentrations of salts.

D. MISCELLANEOUS OBSERVATIONS

Some of the packed-cell pellets created by the centrifugation of cell suspensions containing the salts listed in Tables 7 and 8 were resuspended in water so that their viable cell populations could be determined by plate count assay. The number of viable cells originally introduced could be calculated because the cells in each pellet were obtained from 10 ml of a thawed pellet slurry known to contain 1×10^{12} viable cells per ml. In all cases but one, the suspensions made from salt-treated pellets yielded population estimates that were indistinguishable from those routinely obtained from unprocessed samples of thawed pellet slurry. Thus, the measured distributions of salts and water recorded earlier were obtained with viable cells and not with killed residues. About 90% of the cells exposed to 1.71 N CaI_2, however, were killed. Equally concentrated solutions of $CaCl_2$ and $CaBr_2$ were not toxic under these conditions, and so the antibacterial action of CaI_2 was ascribed to a specific toxicity of this salt and not to a generalized osmotic stress effect.

The optical density of S. marcescens suspensions was measured in exploratory tests. The presence of 1 to 2% NaCl caused a stable and persistent increase in optical density, showing that these cells gave the usual optical effect in the presence of salts.

IV. DISCUSSION

A. PLASMOLYSIS

The simplest interpretation of the data obtained in this study is that S. marcescens was plasmolyzed by concentrated salt solutions, to which the cell walls were permeable but the cell membranes were impermeable. (The cell membrane and its contents will be identified henceforth as protoplasts.) Therefore, when the cells were suspended in concentrated salt solutions, their protoplasts lost water and shrank in order to reduce their internal water activity to match that of the extracellular fluid. As the protoplasts decreased in size, large sections of the elastic cell wall and the cell membrane, tightly bonded together, probably contracted as a unit.[8] Other sections of the cell wall became separated from the underlying cell membrane, and the extracellular salt solution filled the interspace (the plasmolysis space) so created. Thus, the salt solution both plasmolyzed (the cell protoplast) and penetrated (the cell wall). Such reasoning suggests that the water remaining within the protoplasts would be unavailable to salts in plasmolytic concentrations. This inference is contradicted by the data in Tables 4 and 6, which indicate that all the water in packed-cell pellets is accessible to NaCl (and, presumably, the other tested salts) in concentrated solutions.

Dilute salt solutions like 0.1% (0.017 N) NaCl did not plasmolyze the cells (Table 7). The effects of cation adsorption and anion repulsion made it impossible to determine whether all of the salt in the packed-cell pellets could be accounted for by adsorption plus distribution in the between-cell and the cell-wall spaces or if some of the salt had, indeed, entered the cell protoplasts. Only in the case of NaI was there clear evidence that the salt penetrated the cells, because (i) its cations and anions were taken up in equivalent amounts, indicating that ion adsorption and repulsion had not occurred, and (ii) the dilute (0.0171 N) and concentrated (1.71 N) NaI solutions were diluted equally when mixed with S. marcescens cell pellets (Table 8). If one assumes that the concentrated NaI solution secured its water of dilution by plasmolyzing the cells, then the nonplasmolytic NaI solution could only have gained access to this water for its dilution by penetrating the cells.

B. COMPETITIVE DEHYDRATION

The shape of bacterial cells may be due both to the rigidity of their cell walls and to the rigidity of the gel of which their protoplasm may be composed. Swelling and shrinking of microorganisms placed in different solute environments may be due to changes in the extent to which this gel is hydrated.[8] Concentrated salt solutions may have removed water of hydration from the protoplasmic gel of S. marcescens by competitive dehydration in a phenomenon similar to salting-out. Under these conditions the cells might lose water and shrink, but the cell walls might not be able to shrink as much as the protoplasts because of the rigidity of their mucopeptide layer. Consequently, an interspace might be created between the wall and the membrane, and this space would become filled with salt solution. According to this hypothesis the extent of dehydration and consequent shrinkage of the cells depended only on the salt concentration in their environment; in other words, on the relative affinity of the salt and the cell cytoplasm for water of hydration, and not on the permeability characteristics of the cell membrane. Whether this salt was intracellular or extracellular was immaterial. Apparently there was a limit to which the cells could be dehydrated, however, since cells centrifuged out of 4% NaCl or 8% NaCl solutions showed the same net wet weights (Table 5).

A similar hypothesis was proposed to account for the swelling and shrinking of mitochondria in varied solute environments.[19] It would also explain the reported interactions between E. coli and concentrated citrate solutions where an outflow of water from the cells, coupled with the diffusional entry of citrate, was observed but not explained.[16] Also, this theory would account for the observation, not easily reconciled with the plasmolysis hypothesis, that NaI in dilute solution was freely penetrative into S. marcescens, while its concentrated solution was "plasmolytic." Cellular "plasmolysis" has been accepted as evidence of solute nonpenetrativity. The possibility that a solute could be freely

penetrative at low concentration and yet cause cellular dehydration at high concentration is not consistent with the conventional concepts of plasmolysis, but it fits the competitive-dehydration theory nicely.

C. PERMEABILITY ALTERATIONS

It may be that S. marcescens suspended in hypertonic salt solutions can lose only a limited amount of water. If osmotic equilibrium is not reached during this process, the dehydrated cells may admit salt, both to the plasmolysis space and to the protoplasts, until such equilibrium is reached. Salts that were nonpenetrable in dilute solutions could enter the cells in this way. This mechanism could account for (i) the observation that cell pellets were equally dehydrated by 4.3% NaCl and by 8.1% NaCl solutions (Table 5), and (ii) the accessibility of cellular water to 4.4% NaCl (Table 4). It seems implausible that a salt solution would remove all of the free water from the cell protoplasts to the plasmolysis space; it is more probable that some of the salt had entered the water-depleted cells.

Finally, it appears probable that the ability of salts to increase the optical density of cell suspensions is due to the creation of additional light-scattering interfaces through the separation of cell membranes from cell walls. In view of the present findings, it appears much less certain that such an increase in light-scattering is valid evidence of cellular impermeability to salts.

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13. ABSTRACT

The physical interactions between Serratia marcescens and solutions of NaCl, $CaCl_2$, CaI_2, NaI, and (Na_2HPO_4 + NaH_2PO_4) were examined. Dilute (0.017 N) salt solutions did not cause cells to lose water, as evidenced by the unchanged weight of centrifugally packed cells. The cells preferentially adsorbed the cations and repelled the anions of most salts in these solutions. Concentrated (1.71 N) salt solutions markedly reduced the weight and water content of centrifugally packed cells, although these took up considerable amounts of salts. More than 90% of the water in the packed-cell pellets was available for the solution of NaCl at 4.2 to 4.4% concentration. The observation that salts apparently penetrated the entire cells freely and yet caused extensive dehydration was not readily compatible with conventional concepts of solute-induced plasmolysis. Alternative hypotheses to explain the data included the following: (i) The cells lost weight and water to concentrated salt solutions through a nonosmotic competitive dehydration, causing a shrinkage of the protoplasmic gel. The shrinkage of the cell wall was limited because of the rigidity of its mucopeptide layer; therefore, a space appeared between the cell wall and the cell membrane. (ii) Cells may have equilibrated their water activity with that of their environment by two mechanisms: (a) the loss of water by plasmolysis or competitive dehydration, (b) alterations in cell permeability that admitted previously excluded solutes to the cell interior. Possibly the true explanation of the observations reported here will involve elements of all three hypotheses: plasmolysis, competitive dehydration, and permeability alterations.

14. Key Words

Serratia marcescens Inorganic salts Permeability Plasmolysis

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