Electric Conductivity and Internal Osmolality of Intact Bacterial Cells

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Intact cells of Streptococcus faecalis and Micrococcus lysodeikticus were found to have high-frequency electric conductivities of 0.90 and 0.68 mho/m, respectively. These measured values, which reflect movements of ions both within the cytoplasm and within the cell wall space, were only about one-third of those calculated on the basis of determinations of the amounts and types of small ions within the cells. Concentrated suspensions of bacteria with damaged membranes showed similarly large disparities between measured and predicted conductivities, whereas the conductivities of diluted suspensions were about equal to predicted values. Thus, the low mobilities of intracellular ions appeared to be interpretable in terms of the physicochemical behavior of electrolytes in concentrated mixtures of small ions and cell polymers. In contrast to the low measured values for conductivity of intact bacteria, values for intracellular osmolality measured by means of a quantitative plasmolysis technique were higher than expected. For example, the plasmolysis threshold for S. faecalis cells indicated an internal osmolality of about 1.0 osmol/kg, compared with a value of only 0.81 osmol/liter of cell water calculated from a knowledge of the cell content and the distribution of small solutes. In all, our results indicate that most of the small ions within vegetative bacterial cells are free to move in an electric field and that they contribute to cytoplasmic osmolality.

In their well known review of the osmotic properties of bacteria, Mitchell and Moyle (19) presented a persuasive array of evidence for the existence of osmotic barriers to the movement of small molecules into and out of bacterial cells, including both gram-positive and gram-negative types. It is now generally accepted that bacterial cells do possess osmotic barriers and that the major barrier to small, hydrophilic molecules is the cell membrane. Moreover, it is generally accepted that many solutes can enter bacterial cells only by means of transport processes that are coupled to respiration. Despite this apparently settled state of affairs, the results of a number of recent studies suggest that the basic issues regarding bacterial permeability to small solutes, especially small ions, may require further investigations.

For example, Matula and MacLeod (17, 18) found that cells of Pseudomonas aeruginosa and of a marine pseudomonad were freely permeable to NaCl. More recently, Damadian (9, 10, 11) has made a thorough study of ion balances in Escherichia coli cells and has developed the hypothesis that these cells can be viewed as relatively simple, multiphase, ion-exchange systems, and that ions such as K+ and Na+ move into the cells during growth and respiration only as counterions for newly formed polymers or acid products of catabolism. In fact, he proposed that the ion selectivity of the cell is related more to ion-exchange properties of cell polymers than to specific membrane transport reactions, and that terms such as "ion pump" and "active transport" may be misleading.

At present, we are very much in need of a clearer view of the physical states of solutes, especially small ions within bacterial cells. One of the major impediments to obtaining such a view is the almost complete lack of nondestructive techniques for measuring the physicochemical properties of intracellular solutes. These properties can be measured generally only after the solutes have been extracted by damaging cells in some way. Since extraction leads not only to physical damage but usually also to large-scale dilution, there may be radi-
cal alterations of in vivo associations among small molecules, polymers, and water. Thus, there is always at least some ambiguity in the interpretation of experimental results.

With the development of dielectric techniques, it has become possible to make high frequency, dielectric measurements on intact cells, including bacteria. Cell membranes normally act as insulators for low frequency, electric current, but they can be effectively short circuited by use of 50 to 100 MHz current (2, 24). Therefore, with high frequency current, it is possible to measure directly cytoplasmic dielectric properties and so obtain a "glimpse" of the cell interior without inflicting any damage.

Pauly and Schwan (24) have used dielectric techniques to measure internal conductivities of red blood cells and have correlated measured conductivity values with values predicted on the basis of the amounts and types of electrolytes within cells. They concluded that the electrical properties of red cell cytoplasm are essentially the same as those of a concentrated hemoglobin solution. They found in the course of these studies that the mobilities of ions in concentrated protein solutions were reduced to values which were only about 50% of those typical of ions in pure electrolyte solutions. The basis for the reduction appeared to lie in a hydrodynamic interaction between the hydrated ions and the surfaces of protein molecules. Recently, Carstensen, Marquis, and Gerhardt (5) have made a similar study of bacterial endospores. However, they found that measured conductivities of these condensed cells were only about 0.1% of those predicted on the basis of known spore constituents. Thus, it appeared that most of the internal electrolytes of the spores were precipitated or in some other way bound so that they could not move in an electric field.

Red blood cells and bacterial spores are both rather extreme cell types. The former are differentiated and simplified to subserve their gas-transporting functions, whereas the latter are condensed, dormant cells adapted to tolerate severe environmental extremes. In all, it seemed worthwhile to attempt to correlate measured and predicted conductivities of more typical bacterial cells, especially since the correlation could significantly add to our knowledge of the physicochemical states of intracellular solutes.

**MATERIALS AND METHODS**

**Bacteria.** *Streptococcus faecalis* (faecium) ATCC strain 9790 and *Micrococcus lysodeikticus* ATCC strain 4698 were grown in a medium prepared by dissolving 30 g of Oxoid tryptone (Oxo Ltd., London, England) and 1 g of Marmite (a commercial yeast extract from Marmite, Ltd., London, England) in 900 ml of deionized water. Then 10 g of glucose was dissolved in 100 ml of water; both solutions were autoclaved and subsequently mixed to give a medium with initial pH of 7.2. *S. faecalis* was grown statically at 27°C until the phase of declining growth rate, when the culture pH had dropped to ca. 4.7. The cultures contained ca. 2 x 10^9 cells per ml and ca. 0.7 mg of cells (dry weight) per ml. *M. lysodeikticus* was grown aerobically on a rotary shaker at 30°C until growth had nearly stopped. The cultures contained ca. 10^10 cells per ml and ca. 3 mg of cells (dry weight) per ml. Cells were harvested by means of centrifugation in the cold and were washed once with deionized water prior to use.

**Cell volume measurements.** The volume fraction (p) occupied by cells in a suspension was considered to be equal to the dextran-impermeable volume of the suspension. Very high-molecular-weight dextrans (Dextran 2000 of Pharmacia Corp., Piscataway, N.J.) that are completely excluded by bacterial cell walls (26) were used for the measurements. The exact procedures used have been described previously in detail (4, 21).

**Dielectric measurements.** Dielectric measurements in the frequency range 0.5 to 200 MHz were carried out at 29°C with an RX meter (Boonton Measurements Corp., model 250A) following procedures which have been described in detail previously (2, 24). It is possible to calculate the complex, effective, homogeneous conductivity of cells (s_i^*) in a suspension from measurements of the complex conductivity of the total suspension (s*) and of the suspending medium (s_0^*) by means of the equation:

\[
\frac{s^* - s_0^*}{s^* + x s_0^*} = p \frac{s_i^* - s_0^*}{s_i^* + x s_0^*}
\]

(1)

where p is the cell volume fraction and x is a factor related to cell shape. For spherical objects, this factor is 2, and the shapes of both the cocci used in this study were sufficiently close to spherical to allow for use of 2 as a value for x.

The complex conductivity is defined by the equation:

\[
\sigma^* = \sigma + i\omega\epsilon\kappa
\]

(2)

in which \(\sigma\) is the real conductivity, i is the square root of -1, \(\omega\) is the frequency, \(\epsilon\kappa\) is the permittivity of free space (8.854 \times 10^{-12} \text{F/m}) and \(\kappa\) is the relative dielectric constant.

**Analyses of cell solutes.** The amounts of Na^+, K^+, Mg^{2+}, and Ca^{2+} in cells were determined by use of a Perkin-Elmer model 290 atomic absorption spectrophotometer. Initially, cells were digested with 6 N HCl at 100°C for 24 h prior to analysis, but we subsequently found that acidification of cell suspensions to pH 1 with HCl was sufficient to release all of the ions for analysis.
The amount of phosphate in water extracts of cells heated to 70°C for 10 min was determined by use of the Fiske-SubbaRow method (12). Chloride was assayed following the method of Schales and Schales (25). The amounts of ammonium ion in extracts were determined by microdiffusion analysis as described by Conway (7) with boric acid as absorbent and HCl as titrant. Lysine, arginine, aspartate, and glutamate were all assayed by use of specific decarboxylase reactions. Glutamate was also assayed by means of the glutamic-dehydrogenase method (1). Other amino acids were assayed by chromatographing extracts on paper and then isolating the individual compounds for ninhydrin analyses. Protein was measured by means of the Folin reaction (15).

Osmotic measurements. The osmolarities of lysed cell suspensions or of cell extracts were measured by use of a cryoscopic osmometer (Advanced Instruments, Inc., Newton Highlands, Mass.) or a vapor-pressure osmometer (Mechrolab, Mountain View, Calif.). Quantitative plasmolysis techniques for estimating intracellular osmolarities have been described in detail previously (16).

RESULTS

Predicted and measured conductivities.

The frequency dependence of both effective, homogeneous conductivity (σ2) and effective, homogeneous dielectric constant (κ2) for S. faecalis and M. lysodeikticus cells after one wash in deionized water is depicted in Fig. 1. Conductivities of suspending media (σ1) at the time of the measurements were 0.40 mho/m for S. faecalis and 0.11 mho/m for M. lysodeikticus. Low-frequency and high-frequency limits for the conductivity were estimated by use of Cole-Cole plots (27).

Conductivity at low frequency can be attributed mainly to movements of mobile ions within the cell wall space outside the cell membrane (3, 4). The low frequency limit for conductivity of M. lysodeikticus cells (0.27 mho/m) was significantly higher than that for S. faecalis cells (0.06 mho/m), and so, M. lysodeikticus cell walls appeared to contain higher levels of mobile ions than did S. faecalis walls. It is possible to calculate roughly cell wall conductivities by use of a previously derived (3, 4) equation:

\[ \sigma_2 = \sigma_w \frac{1 - p'}{1 + \frac{1}{2}p'} \]  

where σw is the wall conductivity and p' is the fraction of the cell volume taken up by the nonconducting core or protoplast. At low frequency, σ2 for S. faecalis was 0.065 mho/m, and p' was 0.79 as indicated by measurements of dextran-impermeable and sucrose-impermeable volumes which are described later in the paper. The calculated value for σw is 0.43 mho/m. Since this value is only slightly greater than the value of σ2 (0.40 mho/m), the concentration of mobile ions in S. faecalis walls appears to be only slightly greater than that in the suspending medium. Therefore, the number of counterions for fixed charged sites in the polymeric matrix of the wall must be very low. In contrast, a similar calculation for M. lysodeikticus with p' equal to 0.5 indicates a σw value of 0.81 mho/m, which is considerably greater than σ1 of 0.11 mho/m. Thus, M. lysodeikticus walls appear to be loaded with counterions, so much so that the conductivity of the cell wall space is approximately the same as that of the cytoplasm. This observation is in agreement with previous findings (21, 22) that cell walls of M. lysodeikticus are relatively open meshwork structures with sparse cross-linking and high charge density. Since small ions in the environment can diffuse readily into the cell wall water space, it is possible to increase σ2 by increasing σ1. Thus, as we have shown previously (3–5), σ2 is to an extent dependent on σ1. In these experiments, we purposely attempted to keep σ1 as low as possible to minimize the contribution to σ2 of small ions that diffuse into the cell wall water from the medium.

Conductivity for both cell types increases with increasing frequency (Fig. 1). At high...
frequencies, essentially all mobile ions within the cells, including those in the cytoplasm, should move in the electric field. High-frequency limits for conductivity were 0.90 mho/m for *S. faecalis* and 0.68 mho/m for *M. lysodeikticus*. Thus, *S. faecalis* cells appeared to have higher levels of mobile ions within their cytoplasm than did *M. lysodeikticus* cells. Concomitant with the rises in conductivity with increasing frequency, dielectric constants for the cells declined from about 500 to about 60. The decline reflects the short circuiting of the cell membrane.

How do the directly measured conductivity values compare with values calculated from a knowledge of the ion contents of the cells? As the data presented in Table 1 indicate, the major small ion that could conduct current in the type of *S. faecalis* cells we used was K⁺. Other small ions present in significant amounts include Mg²⁺, Na⁺, HPO₄²⁻ and H₂PO₄⁻, lysine cation, Cl⁻, aspartate, and glutamate anions, and Ca²⁺. Bicarbonate and ammonium ions were present in insignificant amounts.

Zwitterionic amino acids with no net charge were not included in the table because they do not conduct appreciable amounts of current at frequencies below about 500 MHz (6). Further, phospholipids were not assayed since it was considered that their relatively large sizes and close associations within the cell membrane would preclude any significant movement in the electric fields applied. *S. faecalis* is a homofermentative, lactic-acid bacterium which produces lactic acid from glucose supplied in the growth medium. However, this acid was leached from the cells during washing with water and so did not contribute to measured cell conductivity. Finally, the contributions of cell polymers and of water to conductivity should be very small at the frequencies used (27).

To calculate predicted cell conductivities, we multiplied measured ion concentrations by values for equivalent conductance at infinite dilution. There was some uncertainty regarding proper equivalent conductances for phosphate and amino acids since their states of ionization

<table>
<thead>
<tr>
<th>Organism</th>
<th>Ion</th>
<th>Conc (eq/liter of cell vol)</th>
<th>Limiting ionic conductance (mho/m)</th>
<th>Predicted conductivity (mho/m)</th>
<th>Measured conductivity (mho/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Streptococcus faecalis</strong>, ATCC 9790</td>
<td>K⁺</td>
<td>0.263</td>
<td>7.35</td>
<td>1.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Na⁺</td>
<td>0.036</td>
<td>5.01</td>
<td>0.18</td>
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<tr>
<td></td>
<td>Mg²⁺</td>
<td>2 × 0.037</td>
<td>5.31</td>
<td>0.39</td>
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</tr>
<tr>
<td></td>
<td>Ca²⁺</td>
<td>2 × 0.004</td>
<td>5.95</td>
<td>0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cl⁻</td>
<td>0.016</td>
<td>7.63</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPO₄²⁻ + H₂PO₄⁻</td>
<td>1.5 × 0.026</td>
<td>8.2</td>
<td>0.32</td>
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</tr>
<tr>
<td></td>
<td>Lysine⁺</td>
<td>0.022</td>
<td>1.5</td>
<td>0.03</td>
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</tr>
<tr>
<td></td>
<td>Aspartate⁻</td>
<td>0.011</td>
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<tr>
<td></td>
<td>glutamate⁻</td>
<td></td>
<td>4.8</td>
<td></td>
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<tr>
<td></td>
<td>Arginine⁺</td>
<td>0.001</td>
<td></td>
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<td><strong>Total</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.08</td>
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<tr>
<td><strong>Micrococcus lysodeikticus</strong>, ATCC 4698</td>
<td>K⁺</td>
<td>0.091</td>
<td>7.35</td>
<td>0.67</td>
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<tr>
<td></td>
<td>Na⁺</td>
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<td>5.01</td>
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<td></td>
<td>Mg²⁺</td>
<td>2 × 0.023</td>
<td>5.31</td>
<td>0.24</td>
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</tr>
<tr>
<td></td>
<td>Ca²⁺</td>
<td>2 × 0.016</td>
<td>5.95</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cl⁻</td>
<td>0.005</td>
<td>7.63</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPO₄²⁻ + H₂PO₄⁻</td>
<td>1.5 × 0.017</td>
<td>8.2</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lysine⁺</td>
<td>0.017</td>
<td>1.5</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Aspartate⁻</td>
<td>0.048</td>
<td>5.9</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>glutamate⁻</td>
<td></td>
<td>4.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Arginine⁺</td>
<td>0.001</td>
<td></td>
<td></td>
<td>2.06</td>
</tr>
</tbody>
</table>
depend on cell pH. The values indicated are based on the assumption that the cell pH was about 6.5. The sum of the predicted conductivity contributions of each of the ions listed as 3.1 mho/m. This value may be about 20% too high because equivalent conductances at infinite dilution are about 20% higher on the average than conductances at the concentrations indicated. Even with these uncertainties, the conclusion seems clear that the measured cell conductivity was less than one-third of the predicted value.

Table 1 also presents data for M. lysodeikticus cells, which have lower ion contents than do S. faecalis cells. Moreover, they contain nearly equal amounts of Na+ and K+. It is difficult to characterize the growth phases of M. lysodeikticus cultures in TGM medium at 30°C because there does not seem to be any extended exponential growth phase. Instead, the doubling time increases continuously during the culture cycle from about 6 to about 30 h. Despite these slow growth rates and the low ion contents of the cells, growth yields were high, up to $1.5 \times 10^{14}$ cells per ml or 3 mg (dry weight) per ml. The cells used for electrical measurements were harvested near the end of the culture cycle, and again measured cell conductivity was only about one-third of the predicted value.

**Conductivity of suspensions of bacteria with damaged membranes.** To gain an appreciation for the effects of cell structure on intracellular conductivity, we treated S. faecalis pellets with 1 ml of butanol per 10-ml pellet to disrupt membranes, measured conductivities of the resulting mixtures at high and low frequencies, and then diluted the mixtures with deionized water and measured the conductivities of the diluted suspensions. To compare conductivities of concentrated and dilute suspensions, we multiplied the measured values by the inverse of the cell volume fraction for each particular suspension. For example, the initial pellets had cell volume fractions of 0.7, and so pellet conductivities were multiplied by 1/0.7. The conductivity of a 1:10 dilution of this pellet would then be multiplied by 10/0.7 to obtain a value for conductivity of the diluted cell contents. Fig. 2 presents log-log plots of conductivities of cell contents against cell volume fractions. At the upper limit for very dilute suspensions, the total conductivity of 3.5 mho/m is slightly above the predicted cell conductivity of 3.1 mho/m. If the 200 MHz curve is extrapolated to a cell volume fraction of one, the conductivity indicated (0.8 mho/m) is nearly equal to the conductivity measured at 200 MHz for intact cells. Thus, it appears that the difference between measured and predicted cell conductivities can be interpreted solely in terms of the behavior of electrolytes in dilute and concentrated mixtures of small ions, soluble polymers, and insoluble polymers.

![Fig. 2. Changes in total conductivity of an aqueous suspension of butanol-treated S. faecalis cells associated with dilution. Cell pellets to which 10% (v/v) butanol was added at ice-bath temperature were considered on the basis of measurements of dextran-impermeable volumes of undamaged cells to have a cell volume fraction of 0.7. Total conductivity was calculated by multiplying measured conductivity by the inverse of the cell volume fraction. Conductivity was measured within 3 h after butanol treatment.](image)
frequency dependence of conductivity for lysed cell suspensions was small compared with that for intact cells, and this finding indicates that butanol effectively destroyed the electrical insulating properties of the cell membrane.

**Osmotic properties of intact cells and cells with damaged membranes.** The low conductivities of ions in concentrated suspensions of butanol-treated cells (or intact cells) could be due to chelation or binding of the ions. Ion-complexing reactions should also lower suspension osmolality. However, as shown by the data presented in Fig. 3, the osmotic activities of solutes in concentrated suspensions of cells with damaged membranes were, if anything, higher than those of the same solutes in dilute suspensions. For these experiments, cell membranes were not damaged with butanol, but instead were disrupted by freezing and thawing the cells six times (S. faecalis) or by heating cells to 70 °C for 10 min (M. lysodeikticus). Conductivity experiments with cells damaged by these means gave results that were similar to those obtained with butanol-treated cells.

If the data of Table 1 are used to predict roughly intracellular osmolalities, values of 560 and 380 mosmol/liter of cell water are obtained for S. faecalis and M. lysodeikticus, respectively. For example, we assumed that all the Mg²⁺ and Ca²⁺ of S. faecalis cells was bound but that all other ions were free. The osmotically active ions would then be (per liter of cell volume): 263 meq of K⁺, 36 meq of Na⁺, 16 meq of Cl⁻, and 26 mmol of phosphate. Ninhydrin analyses and paper chromatography indicated that the cells contained 62 mmol of amino acids per liter of cell volume, predominantly alanine, valine, and leucines in addition to the charged amino acids listed in Table 1. The total is then 403 mmol/liter of cell volume, or 560 mmol/liter of cell water. The values for predicted osmolality are somewhat higher than the highest values shown in Fig. 3. However, if the curves of Fig. 3 are extrapolated to a cell volume fraction of 1, predicted values of 470 and 270 mosmol/liter of cell volume or 650 and 380 mosmol/liter of cell water are obtained for S. faecalis and M. lysodeikticus, respectively. Thus, the discrepancy between predicted and measured values is not great, and there does not appear to be any large-scale complexing of solutes to form osmotically inactive aggregates with low capacities to conduct current.

Internal osmolalities of bacteria have been estimated also from plasmolysis thresholds of intact cells (19). It is possible to obtain quantitative estimates of the extent of plasmolysis by measuring changes in sucrose-impermeable volumes of cells in concentrated sugar solutions. Sucrose is considered not to penetrate the protoplast membrane of nonrespiring cells. Thus, the volume of the cell that is inaccessible to sucrose (which can readily enter the porous matrix of the cell wall) is equal to the protoplast volume plus the volume of cell wall polymer strands. This latter volume is generally small, less than 5% of the total cell volume (4). Figure 4 shows changes in sucrose-impermeable volumes of S. faecalis cells plotted in relation to the inverses of the osmolalities of their suspending media. At low concentrations, sucrose could penetrate into only about 37% of

![Fig. 3. Changes in total osmolality of aqueous suspensions of lysed S. faecalis or M. lysodeikticus cells. S. faecalis cells were lysed by freezing and thawing six times. M. lysodeikticus cells were lysed by heating them to 70 °C for 10 min. Total osmolalities were calculated by multiplying measured osmolalities by the inverse of the cell volume fraction.](http://jb.asm.org/)

![Fig. 4. Changes in sucrose-impermeable volumes of S. faecalis cells related to equilibrium, sucrose osmolalities of suspending media.](http://jb.asm.org/)
the volume of the cell pellets used. Dextran molecules could enter about 20% of the space of these particular pellets, and so the cells occupied about 80% of the pellet volume. The volume of cell wall water, i.e., that available to sucrose but not dextran, is 37 to 20%, or 17% of the pellet volume. It is 17%/80%, or 21% of the dextran-impermeable pellet volume. In other words, cell wall water accounts for about 21% of the total cell volume. Therefore, the protoplast and cell wall polymers must account for about 79% of the cell volume. The osmolality for incipient plasmolysis is between 1.0 and 1.1 osmol/kg (inverse osmolality between 0.9 and 0.9). In more concentrated solutions, the cell protoplasts contracted and their behavior could be described roughly by use of the Van’t Hoff-Boyle relationship: \( V = b = a/\pi \), where \( V \) is the osmotically active volume of the cell, \( b \) is the osmotic dead space, \( a \) is a constant, and \( \pi \) is the osmolality of the suspending medium.

In all, the results indicate an internal osmolality of ca. 1.0 osmol/kg, and this value is significantly higher than that predicted from a knowledge of the cell content of individual solutes or that measured for damaged cells. Similar experiments with \( M. \) lysodeikticus cells indicated an internal osmolality of ca. 0.52 osmol/kg; again, this value is greater than that found for damaged cells.

The most likely basis for the apparently anomalously high values for internal osmolality measured by the quantitative plasmolysis technique is the nonuniform distribution of ions in cells. For example, the data of Fig. 1 indicate that \( S. \) faecalis cell walls contain few mobile ions, and so it appears that the ions of the cell must be concentrated in the cytoplasm. Furthermore, isolated \( S. \) faecalis walls have very low intrinsic osmolalities, essentially zero as measured by cryoscopic osmometry. \( S. \) faecalis cells used in this study were about 72% by volume water, and measurements of dextran-impermeable and sucrose-impermeable volumes indicated that about 21% of the cell volume was wall water. Therefore, approximately 72% to 21%, or 51% of the cell volume is cytoplasmic water. If all the small ions are concentrated in the cytoplasmic water, then the internal osmolality predicted from values given previously becomes 0.81 osmol/kg. This value is still about 20% below the value indicated by the plasmolysis threshold. However, if one considers that mechanical factors and polymers may also contribute to apparent internal osmolality, then it seems likely that the quantitative plasmolysis technique does give at least a rough estimate of the internal osmolality of intact bacterial cells and can be considered as another nondestructive technique for obtaining information on the physical states of solutes within cells.

### DISCUSSION

The experimental results presented in this paper indicate that small ions within vegetative bacterial cells are free to move in an electric field, even though they have reduced mobilities. As indicated by the comparative data presented in Table 2, the ratios of predicted-to-measured conductivity for bacterial cells are approximately equal to the ratio determined by Pauly and Schwan (24) for erythrocytes. Thus, small ions within erythrocytes and those within vegetative bacterial cells appear to have approximately the same relative mobilities and presumably are in similar physical states. Small ions within Ehrlich ascites cells are less restricted in their movements, and measured conductivities are essentially equal to predicted ones. Table 2 also presents data on the relative solids contents of the cells. It can be seen that Ehrlich ascites cells have low solids contents and, therefore, high water contents. Relative mobilities of

**Table 2. Measured cell conductivities compared with predicted values**

<table>
<thead>
<tr>
<th>Cell</th>
<th>Predicted conductivity (mhos/m)</th>
<th>Measured conductivity (mhos/m)</th>
<th>Ratio predicted: measured</th>
<th>Approximate solids content (%) by wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ehrlich ascites tumor cell</td>
<td>1.4</td>
<td>1.3</td>
<td>1.1</td>
<td>15</td>
</tr>
<tr>
<td>Erythrocyte</td>
<td>1.5</td>
<td>0.5</td>
<td>3.0</td>
<td>35</td>
</tr>
<tr>
<td>Streptococcus faecalis</td>
<td>3.1</td>
<td>0.9</td>
<td>3.4</td>
<td>37</td>
</tr>
<tr>
<td>Micrococcus lysodeikticus</td>
<td>2.1</td>
<td>0.7</td>
<td>3.0</td>
<td>36</td>
</tr>
<tr>
<td>Bacillus cereus dormant spores</td>
<td>34.8</td>
<td>0.02</td>
<td>1740</td>
<td>35</td>
</tr>
</tbody>
</table>

*a Data were taken from a paper by Pauly (23).

*b Data were taken from a paper by Pauly and Schwan (24).

*c Data were taken from a paper by Carstensen et al. (5).
intracellular ions can be related to cell water content, as Pauly and Schwan (24) have done. However, the relationship apparently does not apply to dormant spores. The disparity between predicted and measured values for spores is so great that it is most reasonable to interpret it in terms of precipitation or chelation of spore electrolytes.

The physical basis for the low mobilities of ions in vegetative bacterial cells or in concentrated cell lysates can be related in part to viscosity and excluded volume effects. For example, Kolos and Treiber (14) found that conductivities of KCl solutions were decreased when dextrans were added to them. The decrease was about 2% per g of polymer per 100 ml of solution. Conceivably, much of the reduced mobility of intracellular ions may be due to these effects that occur even in solutions of uncharged polymers. It has been suggested (24) that the mobilities of ions moving in the vicinity of charged polymers are reduced still more. For example, Möller, van Os, and Overbeek (20) found a marked reduction in the equivalent conductances of serum albumin counterions (K+, Na+, or Li+) as the protein net charge was increased. As mentioned earlier, hydrodynamic interactions between hydrated ions and the surfaces of protein molecules may also reduce ion mobilities. In agreement with the conductivity results with whole cells, nuclear magnetic resonance studies (8) have shown that intracellular K+ ions have greater "relaxation" times than do K+ ions in ordinary aqueous solutions.

In all, our experimental results indicate that interpretable information regarding the physicochemical states of intracellular ions can be obtained by use of the nondestructive techniques described. Moreover, it appears that the electrochemical behavior of concentrated suspensions of cells with damaged membranes is similar to that of bacterial cytoplasm, and it should be possible to obtain still more information regarding cytoplasmic electrolytes from studies of condensed suspensions of cell components.

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LITERATURE CITED


