PASSIVE ELECTRICAL PROPERTIES
OF MICROORGANISMS

II. RESISTANCE OF THE BACTERIAL MEMBRANE

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ABSTRACT   Studies of the effective, homogeneous, dielectric constants of bacteria are used to show that the resistances of their cytoplasmic membranes are too great to explain the low-frequency conductivities which have been observed for these organisms. This reaffirms the conclusion that at low frequencies the conductivities of bacteria reflect properties of their cell walls. In the organisms studied, the conductivities of the cell wall region are as great as the conductivities of the cytoplasm. This is true even though the ion concentration in the environment is much less than that in the cells. The mobile ions of the wall are presumed to be counterions for fixed charges in this region.

The effective, low frequency conductivities of bacteria have been related to the properties of their cell walls (Carstensen et al., 1965). From the electrical point of view, the bacterial cell may be described as a shelled sphere (model A of Fig. 1). The central core appears to be an insulator at low frequencies because of the high resistance of the cytoplasmic membrane which defines its outer boundary. The shell, corresponding to the cell wall, is highly conducting by virtue of the presence of mobile counterions for the fixed charges in this region.

   Two assumptions are implied in this model for the passive, electrical properties of the bacterial cell. The first of these is related to the resistance of the membrane. If membrane resistance values of the order of 10^{-6}–10^{-5} ohm m² were to be used, it would be possible to explain the observed conductivities of bacteria by a model which completely disregards the presence of the cell wall (model B of Fig. 1). Although these values seem to be unreasonably small in light of known resistances for other biological membranes (Cole and Moore, 1960; Carstensen and Smearing, 1967), no direct evidence has been obtained for bacteria until the present investigation. The second assumption concerns the nature of the charge carriers in the cell wall. There is a continuous, slow leakage of ions from the interior of the cell. Yet it is assumed that these leakage ions diffuse rapidly through the wall and make a

negligible contribution to the ion concentration which must be postulated for the wall to explain its apparent conductivity. Rather it is assumed that the charge carriers in the wall are counterions for fixed charges on the structural molecules of this region.

This investigation was undertaken to demonstrate that for bacteria, membrane resistances are too high to account for their observed, effective conductivities and thus confirm the original conclusion regarding the importance of the cell wall in the low-frequency, dielectric properties of these microorganisms. The primary evidence to be presented is related to the values of the effective, dielectric constants which models A and B predict for the cells. The low values of membrane resistance de-
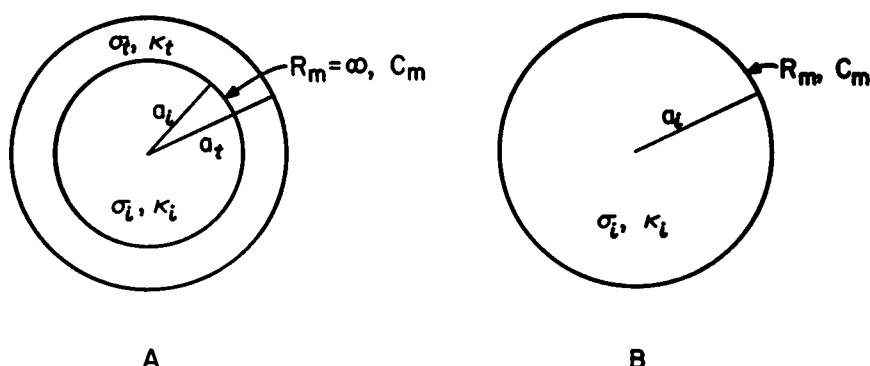

FIGURE 1   Models for the electrical properties of the bacterial cell. Model A assumes an infinite membrane resistance R_m and explains the effective, low-frequency conductivity in terms of the cell wall conductivity σ_t. Model B ignores the cell wall and attributes the low-frequency conductivities to a finite membrane resistance.

manded by model B predict lower values of the effective, dielectric constant than those predicted by model A. Comparison of the ion concentrations in the cytoplasm and in the wall permit us to rule out leakage ions as an important factor in cell wall conductivity.

EXPERIMENTAL PROCEDURE

The organisms studied were *Escherichia coli* strain C1 and an unidentified species of *Micrococcus*. Both organisms were selected for these measurements because they are single spheres. The latter appear in groups of two to four in broth culture but normally separate into single cells after one or two washings. Both organisms were grown in shaken flasks of nutrient broth for 14–18 hr. The growth temperatures were 37°C for *E. coli* and 25°C for the *Micrococcus* sp. The cells were washed twice in distilled water to remove growth medium before they were measured.

Values of the complex conductivity of the bacteria were determined from measurements of aqueous suspensions of the cells. To compute the effective, homogeneous conductivity and dielectric constant for the bacteria one must know the complex conductivity of the suspension and the suspending medium and the volume fraction of the suspension occupied by the cells.

Total cell volume is taken as that fraction of the suspension which is inaccessible to a high molecular weight (150,000) dextran. The difference between this volume and that inaccessible to raffinose is assumed to be the free water space of the cell wall. The basic measuring procedure has been described previously (Carstensen et al., 1965). Both dextran and raffinose concentrations were determined colorimetrically by the phenol–sulfuric acid technique (Dubois et al., 1956). Since measurements with different concentrations of the tracer molecule give the same space values, it can be concluded that there is a negligible adsorption error in the volume determinations. Ratios of raffinose to dextran-inaccessible volumes were 0.78 for *Micrococcus* sp. and 0.82 for *E. coli* C1. These ratios give us our only information about the thickness of the cell walls of these organisms. Measurements of isolated walls of *Bacillus megaterium* (Gerhardt and Judge, 1964) and *Micrococcus lysodeikticus* (Britt and Gerhardt, 1958) indicate that as much as 90% of the cell wall space is water. Hence, for some purposes the cell wall volume may be set equal to the volume of the cell wall water.

Since the cells are spheres, it is possible to obtain precise values for their average size by dividing the total cell volume obtained from dextran space measurements by the total count. Typical values for the radii are 0.70 μ for *E. coli* C1 and 0.40 μ for *Micrococcus* sp.

Complex conductivities σ^+ of aqueous suspensions of bacteria were measured over the frequency range 1-200 MHz with a Boonton Measurements Corporation, Boonton, N. J., RX Meter. Sample holder and calibration techniques were similar to those described by Pauly and Schwan (1965). The effective, homogeneous, complex conductivities σ_2^+ of the bacteria were calculated from the relation (Fricke, 1955)

$$\frac{\sigma^+ - \sigma_1^+}{\sigma^+ + 2\sigma_1^+} = p \frac{\sigma_2^+ - \sigma_1^+}{\sigma_2^+ + 2\sigma_1^+} \tag{1}$$

where σ^+ and σ_1^+ are the complex conductivities[1] of the suspension and a suspending medium, and p is the volume fraction of the suspension which the bacteria occupy. This equation is a valid description of the properties of suspensions up to concentrations of the suspended phase in excess of 0.50 (Fricke, 1924, 1953 *b*). In these measurements, volume concentrations of bacteria of the order of 0.3–0.4 were used. This is well within the limits of applicability of the equation yet high enough that σ_2^+ has a strong effect on the measured conductivity σ^+ of the suspension. There are two reasons for the choice in these studies of the environmental conductivities σ_1 which are shown in Table II. First, Equation (1) provides the greatest accuracy in the determination of the low-frequency conductivity σ_{20} of the particle when $\sigma_1 \rightarrow \sigma_{20}$. Second, the environmental conductivity σ_1 was made much lower than the cell wall conductivity σ_t so that σ_t would be dominated by counterions of its fixed charges rather than by environmental ions (Carstensen et al., 1965). Since $\sigma_{20} \ll \sigma_t$, these two conditions were compatible. On the other hand, the values of σ_1 in these measurements were high enough that leakage of ions from the bacteria was a negligible source of error (Carstensen et al., 1965). The electrical data are reproducible within $\pm 3\%$, the volume data $\pm 5\%$. The effect of these random variations on the standard deviation of the values of σ_2 and κ_2 calculated through Equation (1) are shown by vertical bars in Figs. 3 and 4 (Carstensen and Smearing, 1967).

THEORY

As shown by Fricke (1955) the effective, homogeneous, complex conductivity σ_2^+ of a shelled sphere can be written in terms of the conductivities of the shell σ_t^+

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[1] Here $\sigma^+ = \sigma + i\omega\epsilon_o\kappa$ where σ is the real conductivity, κ is the relative dielectric constant, ϵ_o is the permittivity of free space, and ω is the angular frequency.

and the core $\sigma_i{}^+$:

$$\frac{\sigma_2{}^+ - \sigma_t{}^+}{\sigma_2{}^+ + 2\sigma_t{}^+} = \left(\frac{a_i}{a_t}\right)^3 \frac{\sigma_i{}^+ - \sigma_t{}^+}{\sigma_i{}^+ + 2\sigma_t{}^+} \qquad (2)$$

where a_i is the radius of the core, and a_t is the over-all radius of the sphere. This equation permits us to examine quantitatively the predictions of models A and B for the bacteria. In model B, the simpler case, the outer shell is the cytoplasmic membrane whose thickness, $t = a_t - a_i$, is so small that its properties can be described
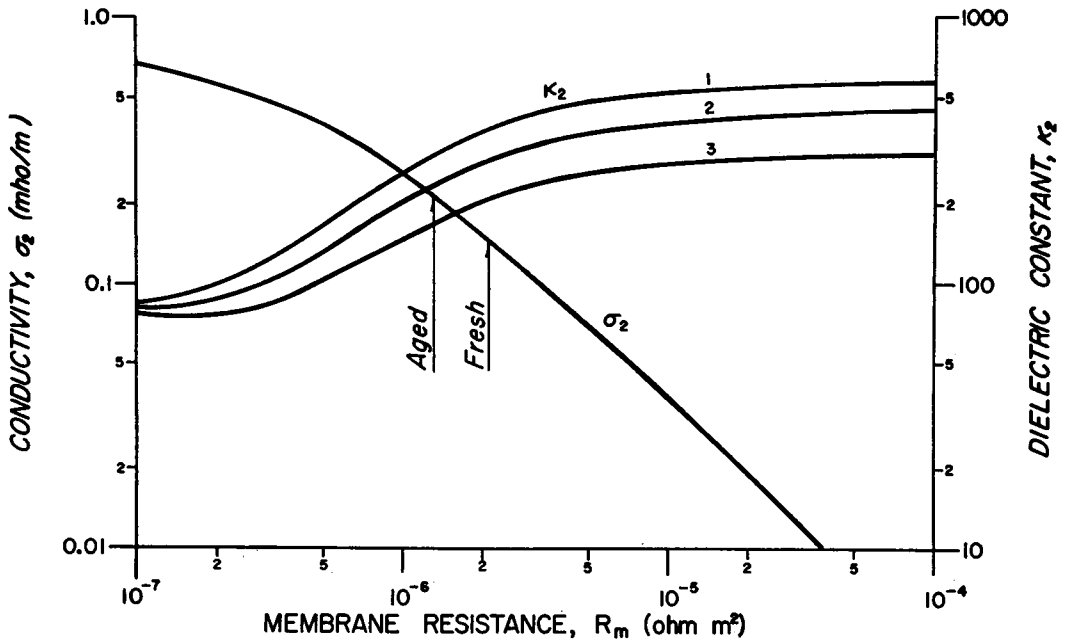

FIGURE 2 Effective, homogeneous conductivity and dielectric constant at 1 MHz as predicted by model B for a cell with a radius of 0.4 μ, internal conductivity of 0.80 mho/m, and an internal dielectric constant of 100. These parameters correspond approximately to the *Micrococcus* sp. of this investigation. Curves 1, 2, and 3 are values of the effective, dielectric constant for membrane capacitances of 0.013, 0.010, and 0.007 farad/m² respectively. Conductivities typical of fresh and aged *Micrococcus* sp. are indicated.

by a membrane resistance $R_m = t/\sigma_t$ and capacitance $C_m = \epsilon_o\kappa_t/t$. The existance of a thin, poorly conducting shell such as this around a highly conducting core gives rise to a dispersion in the effective, homogeneous conductivity and dielectric constant which for bacteria begins at frequencies of the order of 1 MHz and continues over the next two decades. At frequencies of the order of 100 MHz the effect of the shell has vanished and $\sigma_2{}^+ \rightarrow \sigma_i{}^+$. At low frequencies the effective, homogeneous conductivity σ_{20}^B of the membrane-covered sphere can be approximated from Equation (2) (Cole, 1928) as

$$\frac{1}{\sigma_{20}^{B}} = \frac{1}{\sigma_i} + \frac{R_m}{a_i}. \tag{3}$$

In other words, σ_{20}^{B} is an inverse function of membrane resistance. Also from Equation (2) the low-frequency limit of the effective, homogeneous permittivity ϵ_{20}^{B} for the membrane covered sphere can be written

$$\epsilon_{20}^{B} = \epsilon_i + \frac{(\sigma_i a_i C_m - \epsilon_i a_i/R_m)^2}{[\epsilon_i + a_i C_m][\sigma_i + a_i/R_m]^2}. \tag{4}$$

Thus ϵ_{20}^{B} increases with membrane resistance from ϵ_i up to the value which is characteristic of a truly insulating membrane,

$$\epsilon_{20}^{B} = \epsilon_i + \frac{(a_i C_m)^2}{\epsilon_i + a_i C_m}, \text{ (when } R_m = \infty), \tag{5}$$

Fig. 2 illustrates the low frequency predictions of model B for parameters characteristic of the *Micrococcus* sp. used in this investigation.

Equation (2) can also be used to describe model A. In this case the core, which is taken as the membrane-covered cytoplasm, has an effective, dielectric constant and conductivity which would be computed from model B with $R_m = \infty$. The low-frequency conductivity of this new core is 0 as seen from Equation 3, and the effective, homogeneous conductivity σ_2^{A} of the doubly shelled sphere at low frequency becomes

$$\sigma_{20}^{A} = \sigma_t \frac{1 - \rho}{1 + \frac{1}{2\rho}} \tag{6}$$

where $\rho = \left(\dfrac{a_i}{a_t}\right)^3$, i.e., σ_{20}^{A} increases directly with the conductivity σ_t of the cell wall. Again, if the conductivity of this new core is 0, the imaginary part of Equation (2) at low frequencies simplifies to

$$\epsilon_{20}^{A} = \epsilon_t \frac{1 - \rho}{1 + \frac{\rho}{2}} + \epsilon_{20}^{B} \frac{9\rho}{(2 + \rho)^2} \tag{7}$$

where the permittivity of the core (the membrane-covered cytoplasm) is given by Equation (5). Since $\epsilon_{20}^{B} \gg \epsilon_t$, the effective low-frequency dielectric constant of the cell according to model A is determined predominantly by the properties of the core, but, more important in the present discussion, the low-frequency dielectric constant is independent of the conductivity σ_t of the shell. With model A, therefore, changes in σ_{20} will not be accompanied by the changes in κ_{20} which are shown in Fig. 2 for model B.

The effective, homogeneous conductivities and dielectric constants for *E. coli* C1 as determined for frequencies between 1 and 200 MHz are presented in Fig. 3. By direct measurement and reasonable inference, values for the parameters involved in models A and B can be chosen. The radius of the cell is measured as indicated earlier. For these purposes, the volume of the cell wall can be set equal to the volume of the cell wall water. If in so doing the volume of the wall in the model is as much as 20% less than that of the bacterial cell, it simply means that our wall conductivities will be erroneously high by approximately 20% (see Equation 6), but it will not
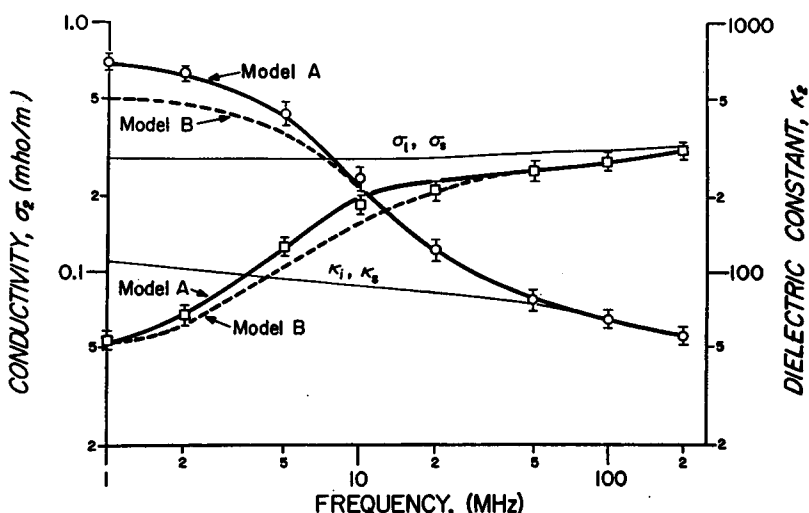

FIGURE 3 Effective, homogeneous conductivity ($\square$) and dielectric constant ($\bigcirc$) of *E. coli* C1. The solid curves represent the predictions of model A, using $a_t = 0.70 \mu$, $a_i = 0.64 \mu$, $C_m = 0.01$ farad/m². The dashed curves are the predictions of model B using $a_i = 0.64 \mu$, $R_m = 11 \cdot 10^{-6}$ ohm m², and $C_m = 0.01$ farad/m². The values of σ_i, σ_t, κ_i, and κ_t used in the calculations are shown by light lines in the figure. Temperature, 25°C.

affect the basic conclusions to be inferred about the properties of the membrane. The cytoplasmic conductivity σ_i and dielectric constant κ_i can be measured directly at high frequencies. Since the conductivity of the cytoplasm is predominantly ionic in origin, there is reason to believe that the σ_i will show very little dispersion. The dielectric constant of the cytoplasm by inference from the properties of other macromolecular solutions probably does undergo a small dispersion. Since $a_i C_m \gg \epsilon_0 \kappa_i$ in Equation 4, this assumption is not critical in the model, but it is probably better to allow κ_i to have the kind of dispersion found in hemoglobin (Oncley, 1938) than to assume that it has the high-frequency value throughout. The dielectric constant κ_t of the cell wall material is arbitrarily taken to be equal to κ_i. The capacitance of the membrane used in the calculations for Fig. 3 is 0.010 farad/m² (1 μf/cm²).

This value appears to be almost a universal property of cell membranes (Schwan, 1957; Fricke et al., 1956) although values as low as 0.0085 farad/m² (Fricke, 1953 a) and as high as 0.013 farad/m² (Schwan and Morowitz, 1962) have been reported. The value of C_m used is critical as is discussed below. In model A the dielectric constant of the wall is set equal to that of the interior and $R_m = \infty$. The only parameters which were chosen to fit the data were σ_i in model A and R_m in model B. Both were chosen to give the observed values of σ_2 at low frequency and held constant at those values for all frequencies. The values of σ_i, σ_t, κ_i, κ_t, thus chosen, are shown by light curves in Fig. 3. The predictions of models A and B for σ_2 and κ_2 are shown by heavy lines in the figure. Model A is completely consistent with the
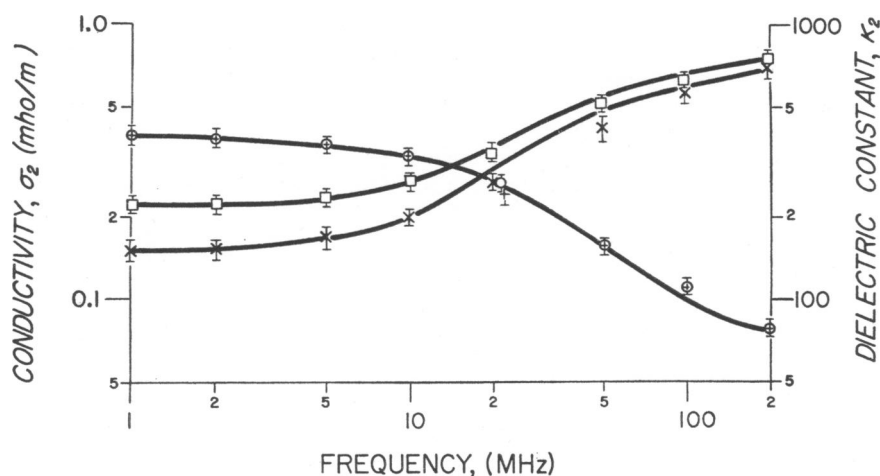

FIGURE 4 Effective, homogeneous conductivity and dielectric constant of *Micrococcus* sp. Data taken immediately after harvesting are represented by the symbols (×) and (+). Observations after the cells had aged for 4 days at 5°C are indicated by (□) and (○). The curves show the predictions of model A using $a_t = 0.38\ \mu$, $a_i = 0.35\ \mu$, $C_m = 0.01$ farad/m², and $\sigma_i = 0.8$ mho/m. For fresh cells, $\sigma_t = 1.1$ mho/m. For aged cells, $\sigma_t = 1.6$ mho/m. Temperature, 25°C.

observations. With the same value of membrane capacitance model B predicts values for κ_2 which are somewhat lower than those observed. However, simply increasing C_m by 30% makes model B fit the data quite as satisfactorily as model A. Since values of C_m as high as 0.013 farad/m² cannot be ruled out, it appears that it will be difficult to choose between the two models from the absolute values of the dielectric data alone.

The best direct evidence on this question was obtained with the *Micrococcus* sp. which was discovered to have a time-dependent low-frequency conductivity. If grown at low temperatures for short periods of time (25° for 14 hr), the low-frequency conductivity immediately after harvesting was found to be as much as a factor of two lower than the conductivity of the same sample after storage for 1 or 2

days at 5°C. Although the initial conductivity appears to depend very critically upon growth conditions, it was possible to obtain four different harvests which showed the same qualitative behavior. A typical experiment is shown by the data points in Fig. 4. In each experiment the dielectric constants of fresh and aged cells were identical within experimental error over the entire frequency range. Only the low-frequency conductivity changed. One may now ask models A and B to predict the changes in the dielectric constant which would be associated with this change in conductivity. It is not necessary to know the absolute magnitude of the membrane capacitance but simply to assume that it does not change with age. First of all, note

TABLE I

COMPARISON OF PREDICTED WITH OBSERVED CHANGES IN DIELECTRIC CONSTANT IN *MICROCOCCUS* SP WITH AGING

The effective, homogeneous conductivity as observed at 1 MHz is given for those samples which showed a change with age. If this change in conductivity is to be explained by model B as a membrane phenomenon, a decrease in dielectric constant is predicted for the aged cells. Model A predicts no change in the dielectric constant. No significant change is observed.

Sample		Effective conductivity, σ_2	Value of R_m required by model B	Drop in effective, dielectric constant predicted by		
				model B	model A	observed
		mho/m	*ohm m² 10⁶*			
No. 1	Fresh	0.15	2.1	100	0	−20
	Aged	0.22	1.2			
No. 2	Fresh	0.14	2.3	50	0	0
	Aged	0.19	1.6			
No. 3	Fresh	0.15	2.1	80	0	−30
	Aged	0.22	1.3			
No. 4	Fresh	0.16	2.0	100	0	−4
	Aged	0.24	1.1			

from the predicted values shown by the solid curves in Fig. 4 that model A (with $C_m = 0.01$ farad/m² and $R_m = \infty$) is completely consistent with the observed dielectric data for this organism in both conditions. One simply says that the conductivity of the wall of the aged cell is greater than that of the fresh cell.[2] Model B, which attempts to explain the effective conductivity of the cell as a manifestation of the properties of the membrane, can be made to fit the data for either fresh or aged cells by proper choice of R_m and C_m. Let us say that the solid curves for the fresh cells in Fig. 4 represent such a choice. If the same membrane capacitance but a

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[2] No attempt can be made here to explain the changes in cell wall conductivity with age. Similar variability in conductivity of bacteria with growth conditions has been observed previously with *Micrococcus lysodeikticus* (Carstensen et al., 1965).

lower membrane resistance were used to explain the higher conductivities of the aged cells, it is apparent from Fig. 2 that model B would call for a drop of about 100 units in the effective, dielectric constant with aging. On the contrary, no significant difference between the effective, dielectric constant of fresh and aged cells has been observed. The results of four such experiments are summarized in Table I.

A further observation which gives indirect support for the concept of a high resistance for the bacterial membrane is related to its permeability to small sugars. Neither raffinose nor sucrose appears to penetrate the membranes of these organisms. Normal erythrocytes have this same property. However, osmium-fixed erythrocytes with membrane resistances in excess of 10^{-4} ohm m^2 are readily permeable to raffinose and sucrose (Carstensen and Smearing, 1967). This is at least an order of magnitude greater than the values of R_m needed for use with model B.

It therefore appears that all available data are consistent with model A and a high resistance for the bacterial membrane. Model B, on the other hand, fails to explain the dielectric data which were characteristic of the aging of *Micrococcus* sp.

ION BALANCE

At frequencies up to 100 MHz the conductivities of the individual compartments of the cell are determined almost entirely by the concentration of mobile ions in these regions.[3] Therefore, granted the validity of model A as a description of bacterial cells, we have in dielectric measurements a unique opportunity to study the distribution of ions in intact living microorganisms. Values of the conductivities of the cytoplasm and cell wall which were necessary to fit model A to the dielectric data for *E. coli* C1 and *Micrococcus* sp. are summarized in Table II. Because of uncertainties in ion mobility and cell wall volume, these values can be used only for rough estimates of ion concentration. However, it is apparent that for both organisms the ion concentrations in the cell wall and cytoplasm are of the same order magnitude even though both are much greater than ion concentrations in the environment of the cells. It is as though these bacteria behave as simple Donnon systems with the mobile ion concentrations determined by fixed charges on the structural molecules of the wall and on the macromolecules of the cytoplasm and with the membrane playing only a secondary role in the ion balance. It should be noted that these cells had been washed in distilled water prior to measurement and at the time of measurement were held at volume concentrations of approximately 0.3–0.4 without benefit of an external energy source. Under these conditions, it would not be surprising to

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[3] Pauly and Schwan (1966) have shown that the effective mobility of ions in the interior of an erythrocyte is somewhat lower than when in free solution. Thus, although the direct relationship between conductivity and ion concentration is a reasonable assumption, the proprotionality factor is uncertain. Mobilities of ions in the wall and cytoplasm, although probably comparable to each other, may be as much as a factor of two lower than the mobilities of the same ionic species in the environment of the cells.

find that the influence of active transport on ion balance is negligible (Schultz and Solomon, 1961).

With this information about the distribution of mobile ions in these bacteria it is possible to rule out leakage as an important contribution to the concentration of ions in the cell wall. As indicated in Table II, the cell wall conductivity may actually exceed that of the cytoplasm. Since there is no mechanical barrier to the passage of ions from the cell wall into the environment, they must be held by electrostatic forces of the fixed charges in the wall.

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TABLE II

INTERNAL AND CELL WALL CONDUCTIVITIES

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Values of σ_i and σ_s which were used in fitting model A to the observed dielectric data are shown for the two organisms of this investigation. Note that in the calculations the cell wall volume was assumed to be equal to the volume of the cell wall water. Since total wall volume is probably 1.1–1.2 times greater than the wall water space, actual wall conductivities will be 10–20% less than the values indicated in the table.

Organism	σ_1 Environmental conductivity	σ_2 Effective, homogeneous conductivity	σ_i Internal conductivity	σ_t Cell wall conductivity
	mho/m	*mho/m*	*mho/m*	*mho/m*
E. Coli Cl	0.04	0.06	0.30	0.30
Micrococcus sp.				
Fresh	0.3	0.15	0.8	1.1
Aged	0.3	0.22	0.8	1.6

CONCLUSIONS

It may be concluded from the dielectric data that the resistance of the bacterial membrane is too high to explain the effective conductivities of these organisms. This supports the conclusion that the low-frequency conductivities of bacteria are determined by the properties of their cell walls. Since the mobile ion concentration is as great in the wall as it is in the cytoplasm, it is possible to rule out leakage as an important contribution to the population of ions in the wall and thus support the postulate that cell wall conductivity is directly related to the concentration of fixed charge in this region.

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