PASSIVE ELECTRICAL PROPERTIES OF MICROORGANISMS

III. Conductivity of Isolated Bacterial Cell Walls

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ABSTRACT The dielectric properties of isolated *Micrococcus lysodeikticus* cell walls have been studied to establish more firmly the view that wall-associated ions play a major role in the conduction of low frequency electric current by intact bacterial cells. The conductivity of isolated walls was found to be about 0.40 mho/m. If counterions associated with fixed, ionized groups in the wall have average mobilities equal to that of sodium ions in free solution, the fixed charge concentration required to account for the measured conductivity is between 75 and 95 meq/liter of wet wall volume. Estimates of the numbers of titratable amino and carboxyl groups in wall polymers indicate that conductivity is more closely related to net wall charge than to total wall charge. The measured wall conductivity was used to predict a value of 0.15 ± 0.03 mho/m for whole cell conductivity. This prediction is close to the measured value of 0.25 ± 0.05 mho/m and it is thought that much of the disparity in values is related to changes in wall structure and composition during the isolation procedures.

INTRODUCTION

To relate the dielectric properties of bacterial cells to their cytological structure, a model has been proposed (Carstensen 1965, 1967) in which the cell is considered to be a three phase structure consisting of a highly conducting cytoplasmic core enclosed by a thin insulating membrane (the plasma membrane) and an external, highly conducting shell (the cell wall). The concentration of mobile ions in the cell wall needed to account for the low frequency conductivity of whole cells was calculated to be almost as great as the cytoplasmic concentration, even though the cell wall is porous and poses no mechanical barrier to diffusion of small ions out into the suspending medium. In fact, the apparent concentration of mobile ions within the wall exceeded the medium concentration in some instances by as much as a factor of ten. This retention of mobile ions within the wall structure suggests that they are serving as counterions for fixed charges there.

To establish more firmly that the cell wall is highly conducting, we have meas-

ured the dielectric properties of isolated, cell-free, *Micrococcus lysodeikticus* walls and have attempted to correlate conductivities with numbers of titratable, fixed, ionized groups. The results obtained with isolated walls could then be used, following the assumptions of the previously proposed model, to predict roughly the electrical properties of whole cells.

MATERIALS AND METHODS

Bacteria

M. lysodeikticus ATCC strain 4698 was grown at 30°C with agitation on a rotary shaker in a medium prepared by adding 30 g Oxoid tryptone, 10 g glucose, 1 g Marmite (a commercial yeast extract from Marmite Ltd., London, England), and 0.5 ml molar phosphoric acid to a liter of distilled water; the final pH of the medium was adjusted to 7.0. The growth period for *M. lysodeikticus* in this medium extended over periods up to 60 hr. The doubling time (in terms of cell dry weight) early in the period was about 6 hr and it increased progressively to more than 30 hr near the end of growth.

Isolation and Purification of Cell Walls

Cultures to be harvested were centrifuged in the cold, the supernatant fluid was decanted, and 4.0 ml chilled 0.05 M phosphate buffer (pH 7.2) per g of pellet weight was added to the cells. The cells were thoroughly resuspended, and 0.1 g extra-fine, homogenizing, glass beads (Scientific Products, Inc., Evanston, Ill.) per g of suspension was added. The suspensions were then placed in a Rosett cooling cell (Branson Instruments, Inc., Stamford, Conn.) immersed in an ice bath, and treated with a Branson sonic oscillator (Model S125) at full power for 20 min. Walls were separated from other cell debris following the general procedure outlined by Salton (1964).

The homogeneity of the isolated wall preparations was evaluated by electron microscopy; many fragments with dimensions of about 1 μ were seen with few smaller fragments and no whole cells. Wall preparations were routinely viewed by phase-contrast microscopy also.

The addition of lysozyme to isolated walls suspended in water resulted in decreases in suspension optical density (700 mμ) of 94–95%. The amount of sedimentable material (23,500 $\times$ *g* for 60 min) after lysozyme treatment weighed less than 3% (on a dry weight basis) of the sedimentable material before lysozyme addition. Absorption-scattering spectra (220–700 mμ) of wall suspensions showed no absorption peaks.

Walls were used shortly after preparation or they were frozen until needed.

Volume Measurements of Whole Cells and Cell Walls

The volume occupied by intact cells in a suspension was taken to be that fraction of the total suspension volume not available to high molecular weight (150,000) dextran. Dextran molecules of this size were considered to be completely excluded by the wall (Britt, 1958; Gerhardt, 1964), and so the dextran-impermeable volume included the volumes occupied by the bacterial protoplast and by its ensheathing cell wall. Details of the techniques for measuring dextran-impermeable volumes and the relative volumes of cells impermeable to other solutes have been described previously (Marquis, 1964; Carstensen, 1965, 1967). With these techniques, whole cells were found to have a volume of 3.95 ml/g dry weight when they were suspended in distilled water.

The same techniques were used with isolated walls. The dextran-impermeable volume of

cell walls was found to be related to the salt concentration of the suspending medium. As shown in Fig. 1, isolated walls shrank when NaCl was added to wall suspensions.

To relate conductivity of isolated walls to that of whole cells, it is necessary to have some estimate of the fraction of the cell occupied by wall. The most reliable method for estimating this fraction involves removing walls from whole cells by treating them with lysozyme and then determining the weight of the remaining protoplasts. It has been reported that lysozyme completely strips the wall from this bacterium (McQuillan, 1960).

Cells suspended in 2 molal sucrose solutions were treated with lysozyme (0.4 mg per ml) until all of them became osmotically sensitive. The resulting protoplasts were fixed with approximately 0.6 M formaldehyde, centrifuged in the cold, and washed twice with distilled water. Fixation and washing resulted in essentially complete leakage of low molecular weight substances from the cells, and so the protoplast weight was corrected for this leakage by add-

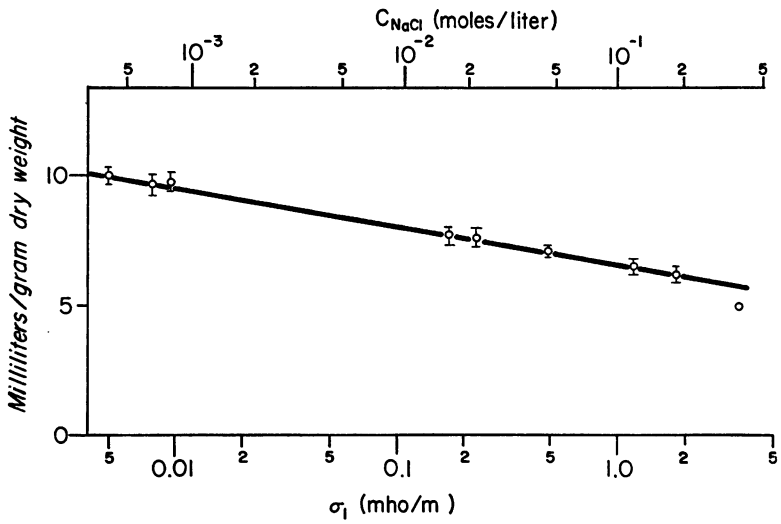

FIGURE 1 Volume of isolated walls of *M. lysodeikticus*.

ing to it the weight of materials extracted from equal numbers of whole cells by heating or by butanol extraction. The values given in Table I indicate that about 35% of the dry weight of this organism is cell wall, and that this fraction is essentially the same for cells harvested early in the culture cycle and those harvested late in the cycle.

The volume fraction of the cell represented by wall comprises the volume fraction occupied by wall structural materials plus the volume fraction occupied by water within wall pores.

The wall structural material, since it is mainly mucopeptide, probably has a specific volume of 0.7 ml/g. Thus, this material, which represents 35% of the cell dry weight should occupy about 0.24 ml per dry gram of cells. The portion of the volume of wet cells occupied by wall solids is relatively small—approximately 0.06 ml per ml of cells.

The volume fraction of wet cells represented by wall water can be estimated from the volume of the cells available to compounds such as sucrose and phosphate, which are small enough to enter wall pores but cannot penetrate the protoplast membrane of nonrespiring cells. About 0.45 of the cell volume was found to be accessible to phosphate, and this value agreed with an average sucrose space of about 0.40. Therefore, the volume fraction of the cell

occupied by wall appeared to be 0.06 plus 0.43 (the average of the phosphate and sucrose spaces) or 0.49.[1]

An alternative method yields nearly the same value for the volume fractions of the cell occupied by wall and protoplast. It is possible to measure the ratio V_W of wall water space to total volume for isolated walls. When sucrose was used to measure the total water space and dextran to measure the wall space, V_W was 0.80 $\pm$ 0.10. The sucrose-available space for the intact cell was found to be 0.40. If we assume that the ratio of wall water volume to total wall volume for isolated walls is the same as that for the walls of whole cells, the wall fraction of the whole cell is 0.50.

Since the cell radius is 0.4 μ, the wall thickness must be about 800 A in a living cell. This value is about twice the value of 400–500 A (Salton, 1964) obtained by measuring electron micrographs of dried, fixed, sectioned cells.

TABLE I

LOSS OF WALL MATERIALS FROM LYSOZYME-TREATED *MICROCOCCUS LYSODEIKTICUS* CELLS

Material	Per cent cell dry weight	
	Culture no. 1*	Culture no. 2*
	%	%
Whole cells	100.0	100.0
Fixed, washed protoplasts	47.2	47.8
Materials released from whole cells by 10% butanol	17.5	16.4
Sedimentable debris from butanol extraction	81.2	83.1
Material released from whole cells at 70°C for 10 min	18.0	16.8
Sedimentable debris from heat extraction	82.3	78.8
Wall material (average, by difference)	35.0	35.6

* At harvest, culture no. 1 contained 1.21 mg cells, dry weight, per ml; culture no. 2 contained 3.76 mg cells, dry weight, per ml. Doubling times, in terms of dry weight at time of harvest, were about 7 hr for culture no. 1 and about 32 hr for culture no. 2.

Acid-Base Titrations of Isolated Walls

Suspensions containing 10–16 mg (dry weight) isolated walls per ml were titrated by sequential addition of standardized solutions containing HCl, NaOH, or KOH. The pH after each addition was measured with a Beckman model G pH meter (Beckman Instruments, Inc., Palo Alto, Calif.). Separate samples were used for acid titration and for base titration. Degassed, CO_2-free solutions were used for the titrations, and during the procedure, a dish of concentrated NaOH solution was placed in the meter chamber to depress the CO_2 level in the air. The pH of alkaline solutions was measured with a Beckman E-2, blue-glass electrode to reduce sodium-ion errors. Typical titration curves are shown in Fig. 2. Electrode responses in extremely acid or alkaline solutions were calibrated with standard acid and base solutions.

When NaCl was added to suspensions of extensively washed walls, there was a rapid drop

[1] The value of 0.49 may be slightly too low because we did not take into account water of hydration in the wall or water otherwise unavailable to small molecules. However, there may also have been some adsorption of the solutes used to measure the wall water space, and in all, we feel that 0.49 is sufficiently close to the true value for the purposes of this paper.

in suspension pH. Although measured pH decreases were as great as 2.85 pH units, the net number of protons released into the medium was small and could be accounted for by displacement of protons from less than 1% of the ionizable groups.

The total numbers of acidic and basic groups that could be titrated did not appear to be greatly influenced by the ionic strength of the medium but, of course, the apparent pK values of the groups were affected. The amino acids making up the peptide portion of the mucopeptide of this bacterium have been found to be: L-alanine, D-alanine, D-glutamic acid, L-lysine and glycine (Salton, 1953; Perkins, 1959; Czerkawski, 1963). A recently proposed structure for the peptide indicates the major ionizable groups to be the alpha-carboxyl groups of alanine and glycine and the epsilon-amino group of lysine (Mirelman, 1966). The curves of Fig. 2 suggest that proton binding at alkaline pH can be mainly attributed to epsilon-amino groups with some contribution by alpha-amino groups including amino groups of unacetylated hexosamine moieties; while proton binding at acid pH appears to be mainly due to

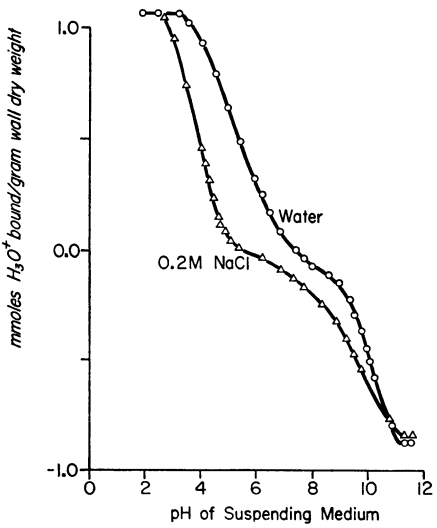

FIGURE 2 Acid-base titration of isolated *M. lysodeikticus* walls. Walls were initially suspended in distilled water (○—○) or 0.2 M NaCl solution (△—△).

alpha-carboxyl groups. Similar titration curves have been obtained by Czerkawski, Perkins, and Rogers (1963), but their curves show fewer groups titrating in the pH range 5–9.

The behavior of isolated *M. lysodeikticus* walls suggests that they bind cations; a similar binding of cations by isolated *Staphylococcus aureus* cell walls has recently been reported by Cutinelli and Galdiero (1967). Thus a reliable isoionic pH for the walls could not be determined. Also, there is a difficulty in assigning intrinsic pK values to titratable groups because the walls are insoluble. In view of these difficulties, we chose to use the initial pH values (about 7.5 for walls in water or 5.4 for walls in 0.2 M NaCl solution) as zero points; groups titrating in more alkaline media were considered to be amino groups, while those titrating in more acid media were considered to be carboxyl groups. This choice clearly affects the relative numbers of positive and negative groups calculated from the titration curves, but the uncertainty is not great.

A more important uncertainty in the determination of fixed charges in the wall arises in connection with the methods available for determination of cationic sites. Base titrations of wall preparations showed varying degrees of irreversibility. In some cases, base titrations

indicated that exposure of walls to pH 12 resulted in an apparent doubling of the number of titratable amino groups. With other preparations, the effect was much less pronounced. Estimates of cationic sites from anionic dye binding give considerably lower numbers than are deduced from base titrations. Czerkawski, Perkins, and Rogers (1963) found that only about one-third of the titratable amino groups bound orange G at pH 2.2. We found even less binding of orange G, but did find that a smaller anionic dye, naphthol yellow S (molecular weight of 359 compared with 452 for orange G) was bound to a greater extent than orange G (0.15 vs. 0.10 mmole dye bound per g walls, dry weight). Thus, the dyes react with only a fraction of the amino groups, and therefore set a lower limit to the numbers of cationic sites. Exposure of the sample to high pH may alter the wall structure and expose cationic sites which are not available in the native state. Thus base titration probably sets an upper limit for the number of positively charged groups in the normal wall. All factors considered, however, the values obtained by base titration are probably the most reliable.

Czerkawski, Perkins, and Rogers (1963) found that the extent of binding of cationic safranine was in agreement with the numbers of carboxyl groups determined by acid titration. Further, the data of Britt and Gerhardt (1958) on the numbers of cationic lysine molecules bound at physiologic pH by isolated *M. lysodeikticus* walls indicate essentially the same value for the concentration of anionic groups as that obtained by the other two methods.

Dielectric Measurements

Dielectric measurements of suspensions of both intact cells and isolated cell walls have been made over the frequency range 1–200 MHz by techniques which have been described previously (Carstensen, 1967). Typical data are shown in Fig. 3. As shown by Fricke (1955), the complex conductivity σ^+ of a suspension of particles with complex conductivity $\sigma_2{}^+$ in a suspending medium with complex conductivity $\sigma_1{}^+$ is given by

$$\frac{\sigma^+ - \sigma_1{}^+}{\sigma^+ + x\sigma_1{}^+} = p\,\frac{\sigma_2{}^+ - \sigma_1{}^+}{\sigma_2{}^+ + x\sigma_1{}^+} \qquad (1)$$

where p is the fraction of the suspension volume occupied by the particles, and x is a factor related to the shape of the particles. For spheres, x is 2. Because intact cells are spheres, it is comparatively straightforward to calculate their effective, homogeneous, dielectric constants and conductivities from a knowledge of the dielectric properties of the suspension (Fig. 3*a*) and the suspending fluid, together with data on the volume fraction of cells in the suspension. At frequencies below 5 MHz and with cells suspended in media with $\sigma_1 \simeq 0.1$ mho/m, the effective, homogeneous conductivities of the cells were found to range from 0.18–0.33 mho/m, depending on the growth conditions of the cultures before harvest. Cells harvested from cultures approaching the stationary phase of growth were found to have the highest conductivities.

As indicated in Fig. 3*b*, suspensions of isolated cell walls showed no dispersion in environments with conductivities comparable to that of the wall material itself ($\sigma_2 \simeq \sigma_1 \simeq 0.44$ mho/m).[2] However, when $\sigma_2 \gg \sigma_1$ the suspensions exhibit the kind of Maxwell-Wagner relaxation which is characteristic of inhomogeneous dielectrics. The low frequency conductivity of suspensions is very much dependent on the shape of the suspended particles when $\sigma_2 \gg \sigma_1$. Since the shape of the wall fragments could not be characterized with any precision, it was

[2] There is preliminary evidence of a dispersion in the dielectric constant and conductivity in the frequency range below 1 MHz for whole cells (Schwan 1957; Einolf 1967) and cell walls as well. No attempt has been made to consider this phenomenon in the present investigation.

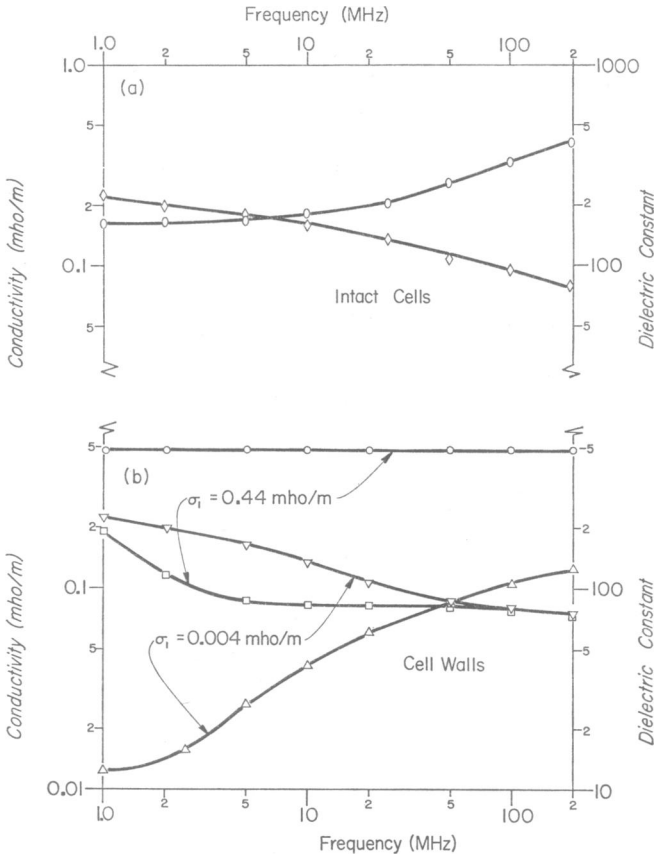

FIGURE 3 Typical dielectric measurements. (*a*) Suspensions of intact *M. lysodeikticus*. Dielectric constant (◇) and conductivity (0) with environmental conductivity $\sigma_1 = 0.12$ mho/m, volume fraction of cells $p = 0.45$, temperature 25°C. (*b*) Suspensions of isolated cell walls of *M. lysodeikticus*. In measurements with environmental conductivity approximately equal to wall conductivity ($\sigma_1 = 0.44$ mho/m, $p = 0.24$), the dielectric constants (□) and conductivities (○) show no sign of Maxwell-Wagner dispersion. With $\sigma_1 = 0.004$ mho/m, $p = 0.30$, the dielectric constants (▽) and conductivities (△) show clear evidence of Maxwell-Wagner dispersion. Temperature 25°C.

impossible to obtain useful estimates of the conductivity of the wall material itself from the low frequency data. Fricke (1953) showed that the limiting value of the conductivity of a suspension of ellipsoids at high frequencies becomes

$$\sigma_\infty = \sigma_2 + \frac{(\sigma_1 - \sigma_2)(1 - p)}{1 + \frac{p}{3}\sum_{\alpha}^{3}\frac{\epsilon_1 - \epsilon_2}{\epsilon_2 - x_\alpha \epsilon_1}} + \frac{\frac{p}{3}(1 - p)(\sigma_2 \epsilon_1 - \sigma_1 \epsilon_2)(\epsilon_1 - \epsilon_2)\sum_{\alpha}^{3}\frac{x_\alpha + 1}{(\epsilon_2 + x_\alpha \epsilon_1)^2}}{\left(1 + \frac{p}{3}\sum_{\alpha}^{3}\frac{\epsilon_1 - \epsilon_2}{\epsilon_2 + x_\alpha \epsilon_1}\right)^2} \quad (2)$$

where x_α is a function of the shape of the suspended particle. For the case $\epsilon_1 \simeq \epsilon_2$, which applies to cell wall fragments, this equation reduces to

$$\sigma_\infty \cong \sigma_2 p + \sigma_1(1 - p). \tag{3}$$

Thus, at high frequencies[3] it is possible to obtain precise values for the conductivity of the wall fragments even though it is impossible to characterize their shape. Since the conductivity of the wall material is determined almost entirely by its mobile ions and since the concentration of other solids which might contribute relaxation effects is very low, the cell wall material itself probably has little dispersion in the 1–100 MHz frequency range. The low frequency data are difficult to interpret quantitatively but they are not inconsistent with this assumption.

Volume concentrations of the suspended phase were between 0.2 and 0.4 for measurements both of the cell wall fragments and of whole cells.

RESULTS AND DISCUSSION

Cell Wall Conductivity and Charge

The minimum environmental conductivity σ_1 that could be used in measuring intact cell conductivity σ_1 was set by leakage of ions from the cells. In practice, the lower limit of σ_1 easily obtainable with bacteria was about 0.05 mho/m. Loss of ions from isolated wall fragments into the suspending medium was less extensive, and it was possible to measure wall conductivity over a range of environmental conductivities from 0.005–3 mho/m.

Cell wall fragments from cells harvested near the middle of the culture cycle were suspended in a series of NaCl solutions. The suspension conductivities (σ) were measured at frequencies of 100 and 200 MHz. These conductivities, together with the conductivities σ_1 of the suspending media and the volume fractions (p) of walls in the suspensions, were used in equation 3 to calculate the conductivities σ_w of the wall material.[4] The results are shown in Fig. 4. The wall preparations were stable for at least 10 days; the reproducibility of the measurements from day to day and from one preparation of walls to the next is indicated by the vertical bars in the figure. The dependence of σ_w on σ_1 is similar to that found for whole *M. lysodeikticus* cells (Carstensen, 1965).

In the first paper of this series, it was postulated that the low frequency, effective, homogeneous conductivity σ_2 of intact cells was directly proportional to the conductivity σ_w of the cell walls (Carstensen, 1965). It was further postulated that σ_w was a function of the fixed charge concentration c_f^w in the wall and the environmental ion concentration c^0

$$\sigma_w \simeq c_f^w u^w [1 + (2c^0/c_f^w)^2]^{1/2} \tag{4}$$

[3] The relaxation frequency apparent in Fig. 3*b* is about 10–20 MHz. Thus equation 3 is applicable at frequencies above 100 MHz.

[4] Note that σ_w is used for wall conductivity and σ_2 for effective, homogeneous conductivity of the intact cell, even though they may both have been determined from experimental results by use of equations 1 or 3.

where u^w is the mobility of ions in the wall. The data of Fig. 4 indicate that isolated walls behaved similarly. Actually, since the walls shrank by a factor of 0.5 in volume as the environmental NaCl concentration was increased over the range shown in Fig. 4, $c_f{}^w$ was probably a function of c^0. If the number of fixed charged groups does not change significantly when the wall shrinks, then

$$c_f{}^w = c_{f0}^w \rho_0/\rho \tag{5}$$

where ρ_0 is the wall volume (per dry g of wall) at very low values of c^0, ρ is the wall volume at higher values of c^0, and c_{f0}^w is the concentration of fixed charges in the

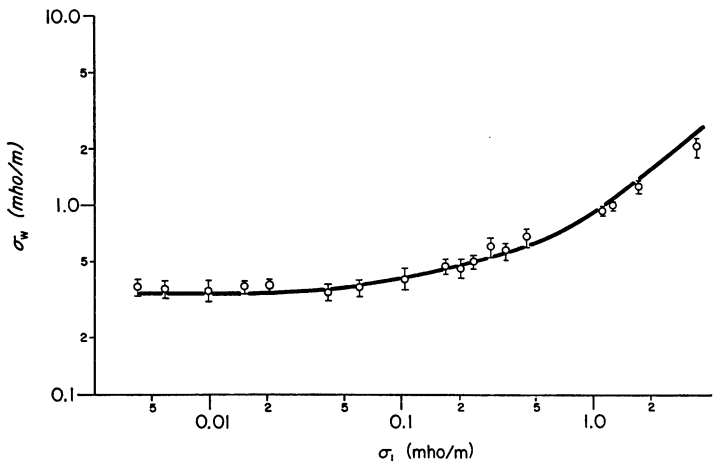

FIGURE 4 Conductivities of isolated cell walls of *M. lysodeikticus*. The environmental conductivity σ_1 can be related to salt concentration c^0 as illustrated in Fig. 1.

wall at low c^0. With this modification, equation 4 becomes

$$\sigma_w \simeq u^w c_{f0}^w (\rho_0/\rho)[1 + (2c^0\rho/c_{f0}^w\rho_0)^2]^{1/2}. \tag{6}$$

The solid curve in Fig. 4 is a plot of equation 6 with c_{f0}^w equal to 90 meq/liter and the mobility of wall counterions u^w equal to that of sodium ions in free solution (4.5 mho/m/equivalent/L). Wall volumes were taken from Fig. 1. It will be noted that c_{f0}^w appears twice in equation 6. Thus, the fitting of this equation to the data of Fig. 4 provides two independent estimates of the fixed charge concentration in the cells. In effect, one value comes from the absolute magnitude of σ_w and the other from the location of the change in slope of the σ_w vs. σ_1 curves. As seen from Fig. 4 these two values agree within the limits of experimental error. The major sources for uncertainty here are in the errors associated with curve fitting near the region of gradual inflection and the need to assume a value for counterion mobility. The error associated with curve fitting is about $\pm 20\%$; the basis for the choice of

a value for counterion mobility has been discussed earlier (Pauly, 1966; Carstensen, 1967).

Estimates of total and net wall charge based on the numbers of titratable groups in isolated wall fragments are summarized in Table II. The fairly wide range of values given reflects mainly differences in titration behavior of walls isolated from cells in different stages of the culture cycle. We found that walls from cells harvested early in the culture cycle were more dense and had more titratable carboxyl groups per unit volume, but lower conductivity than walls from cells harvested from cultures approaching stationary phase of growth. Table II also contains values showing the range of fixed, wall charge estimated from conductivity measurements. It will be recalled that base titrations provide an upper limit for the numbers of amino

TABLE II

CELL WALL CHARGE ESTIMATED FROM CONDUCTIVITY AND FROM ACID-BASE TITRATION

	meq/L
Titration*	
COO^-	121–168
NH_3^+	87–100
Total‡	217–256
Net‡	24–82
Conductivity	75–95

* The number of titratable groups per g dry weight of wall were converted to meq per liter of wall volume by use of the values for wall volume given in Fig. 1.

‡ Total and net charge refer to the range of values obtained with individual wall preparations and not to the sums and differences of the highest and lowest values for amino and carboxyl groups.

groups in the cell wall. Thus net charges in the native wall before exposure to high pH may be higher than indicated in Table II.

It appears that wall conductivity is more closely related to net charge than to total charge. Molecular models of wall mucopeptide structure, constructed according to the formula given by Mirelman and Sharon (1966), show that positively and negatively charged groups may lie closer to each other than the Debye length and so may act as fixed counterions for each other. Recently it has been found (Marquis, 1968) that the compactness of *Bacillus megaterium* cell walls can be related to electrostatic interactions among charged groups in mucopeptides, and this finding supports the view that extensive intramolecular neutralization of charge can occur in these flexible polyampholytes. The mobile ions in the wall must then be counterions for those fixed charges without fixed counterions. In all, it appears that a knowledge of wall conductivity will be of use in determining the physical structure of bacterial cell walls.

Conductivity of Intact Cells Related to Wall Conductivity

The curves of Fig. 3*a* indicate a distribution of relaxation frequencies in the region of 20–40 MHz for whole *M. lysodeikticus* cells. As shown in earlier studies (Pauly, 1962; Carstensen, 1967), this relaxation is related to the presence of the plasma membrane which can be characterized by a capacitance of 0.01 farad/m^2 and an essentially infinite, membrane resistance. At frequencies below 1 or 2 MHz the only structure involved in conduction of current in the whole bacterial cell should be the cell wall. As discussed previously (Carstensen, 1965), it should be possible to relate the effective, homogeneous conductivity of the cell to the wall conductivity as follows

$$\sigma_2 = \sigma_w \frac{1 - p'}{1 + \frac{1}{2}p'} \tag{7}$$

where p' is the fraction of the cell volume taken up by the nonconducting core, or protoplast.[5]

The wall volume fraction as shown in Materials and Methods is approximately 0.50. Thus $p' = 0.50$.

TABLE III

LOW FREQUENCY, EFFECTIVE, HOMOGENEOUS CONDUCTIVITY OF WHOLE *M. LYSODEIKTICUS* CELLS PREDICTED FROM THE CONDUCTIVITY OF ISOLATED CELL WALL FRAGMENTS

	mho/m
Prediction	
σ_w (cell wall conductivity)	0.40 ± 0.04
p'	0.50 ± 0.05
σ_2 (equation 7)	0.15 ± 0.03
Observation	
σ_2	0.25 ± 0.05

All measurements are with environmental conductivity of 0.1 mho/m, which corresponds to approximately 10 mM NaCl. The calculations of values for p' is discussed in the text. All values shown are averages of those obtained with materials from cultures in the early stages of the culture cycle and cultures in the late stages of the cycle.

In Table III, average values for cell conductivity derived from a knowledge of σ_w and p' are compared with average values measured with whole cells. Predicted and observed values for σ_2 agree roughly, even though the predicted value is significantly less than the measured value. The experimental factors that may contribute to this disparity include: loss of wall materials during the isolation procedure, changes in the physical structure of the wall following rupture, tight binding of

[5] No bacterial capsules were observed with these cells.

ions by wall fragments, and the presence of ions more mobile than sodium ion in the walls of intact cells.

We mentioned previously that walls obtained from cells harvested late in the culture cycle were found to have higher σ_w than those obtained from cells harvested early in the cycle. The data presented in Fig. 5 show that the effective, homogeneous, low frequency conductivity of whole cells also increased as the culture cycle progressed; and these observations support the proposed model for the bacterial cell as a conductor, especially in view of the finding (Table I) that the fraction of the cell occupied by lysozyme-sensitive wall material did not change appreciably. Isolated walls from cells in the early stages of culture growth were relatively dense in terms of the dry weight to wet weight ratio, and it may be that internal charge neutralization is more extensive, and electrostatic repulsion reduced, in these walls. Further studies of the chemical and physical make-up of bacterial cell walls are clearly indicated.

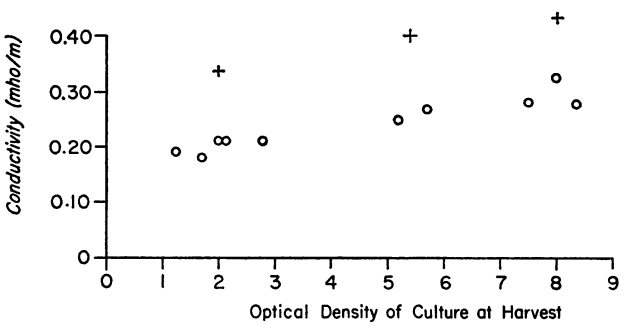

FIGURE 5 Effect of age of culture on conductivity of *M. lysodeikticus* cell walls. Isolated cell walls (+), intact cells (○).

CONCLUSIONS

The high conductivities for cell walls predicted by observations of intact cells have been confirmed by direct observations of isolated bacterial cell walls. The numbers and sign of fixed charge sites in the wall structure are of the correct order of magnitude to explain the wall conductivity as a manifestation of the presence of mobile counterions in the wall water space.

In this way the present study has provided strong support for the basic model which has been proposed to explain the dielectric properties of bacteria. There are small discrepancies between charge estimates by acid-base titration and by conductivity measurement and between the values of the conductivities of isolated and of intact cell walls. However, these differences probably point to the eventual usefulness of dielectric techniques in studying the characteristics of intact cells rather than to a weakness in the model.

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