ELECTRICAL PROPERTIES OF THE MEMBRANES OF THE PLEUROPNEUMONIA-LIKE ORGANISM A 5969

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ABSTRACT The electrical properties of the pleuropneumonia-like organism A 5969 have been determined over the frequency range from 0.5 to 250 Mcps. The frequency dependence of the dielectric constant and conductivity of PPLO suspensions is completely consistent with the existence of a membrane. The PPLO has an internal conductance which in part reflects its ionic equilibrium with normal nutrient and macromolecular constituents. But it is fairly independent from variation in external ionic strength.

Unlike the bacteria, pleuropneumonia-like organisms do not possess a rigid cell wall but thin section electron microscopy on some species has indicated a three-layered structure at the surface similar to the reported membranes of many other systems. In attempting a detailed characterization of *Mycoplasma gallisepticum* A 5969 it became a matter of interest to establish electrical as well as electron optical criteria for the existence of a membrane.

Measurements of the electrical impedance of suspensions of cells are particularly good evidence for the presence of a membrane and its capacitance per unit area (see for example Schwan 1957; Pauly and Schwan, 1959). The capacitance C_M in turn may be interpreted by use of the usual relationship between the membrane thickness d and the dielectric contant of the membrane material ϵ_M

$$C_M = \epsilon_r \cdot \epsilon_M / d \tag{1}$$

($\epsilon_r = 8.84 \times 10^{-14}$ if C_M in farads/cm^2, d in cm). The value of ϵ_M is probably between 10 and 20 (Schwan and Cole, 1960). Numerous determinations of membrane capacitances of biological cells have been carried out during the past four decades as summarized by Cole, 1942; Cole and Curtis, 1950; Schwan and Cole, 1960. The material includes erythrocytes, leucocytes, marine eggs, muscle fibers, ganglion cells, squid and other axons, plant cells and more recently bacteria and mitochondria (Pauly, Packer, and Schwan, 1960). In most cases similar membrane capacitance values near 1 μf/cm^2 have been observed, suggesting a similar membrane thickness and molecular structure.

We have measured the dielectric constant and the conductivity of various suspensions of a pleuropneumonia-like organism (PPLO) over the frequency range from 0.5 to 200 megacycles per second (Mcps). The analysis of the experimental data was performed, using an appropriate theory of the frequency dependence of the electrical properties of a suspension of membrane-covered spheres. The existence of a membrane was readily observed. The magnitude of its capacitance is near the 1 μf per cm^2 of membrane surface observed for other membranes. Similarly we have also determined the electrical conductivity of the interior of the PPLO. Its value is fairly independent from that of the medium in which the cells were suspended. From the point of view of electrical properties PPLO appear to resemble many other biological systems that have been studied.

PREPARATION

The method of cell growth and harvesting cells has been previously reported (Morowitz *et al.*, 1962). In these studies we used the same Difco tryptose medium and harvested cells by centrifuging from a 48 hour culture and washing with fresh medium. The final pellets were stored at slightly over 0°C prior to taking electrical measurements.

The cells were concentrated immediately before measurements were taken. They were repeatedly washed in the suspending medium using a Servall centrifuge at a speed of 17,000 RPM until equilibrium between cell interior and exterior was established. The electrical conductivity of the suspending medium was determined before a suspension was prepared and compared with that of the supernatant collected after centrifugation in order to check the amount of ionic leakage across the membrane. The suspensions proved sufficiently stable to permit measurements without large drift after two or three washings. Preparations of the microorganism in normal nutrient and in threefold diluted nutrient solution were investigated.

Pellet volume fractions, *p*, were obtained with the centrifuge running at 14,000 RPM. Final values were approached after 2 hour runs. Independent volume fraction determinations were available from low-frequency conductance of suspension and suspending medium (see Appendix). The centrifuge data were always slightly higher (about 10 per cent relative) than the electrical data. This has been observed also with other cellular structures and is likely to reflect the impossibility to completely pack cells with the centrifuge. Averages of centrifuge and electrical volume fraction data were used in the analysis. Thus the relative error in volume fraction is smaller than 5 per cent.

Cell size determinations have been carried out by two procedures. Electron micrographs of osmium-fixed cells (Morowitz *et al.*) indicate that the cells are spherical and have an average diameter of 0.25μ. The extremes of observed cell size range from 0.15μ to 0.50μ. The cell sizes have been independently measured by filtration through Millipore filters. Using filters of pore diameter of 0.30μ over 90 per cent of the viable cells pass through the filter. Using filters of 0.22μ pore diameter less than 0.1 per cent of the viable cells pass through the filter. The filter pore sizes were calibrated with a series of known virus particles.

MEASUREMENTS

The electrical properties were measured over the frequency range from 0.5 to 250 Mcps with a commercially available instrument (RX-meter, type 250-A, Boonton Radio Corporation, Boonton, N. J.). This instrument is designed to measure the equivalent parallel resistance R_p (in ohms) and the parallel capacitance C_p (in $\mu\mu$ farads) of the sample. It consists of a Schering bridge together with associated oscillator, amplifier, null detector, and power supply. The cell which was filled with the microorganismal suspensions requires less than a 0.1 cc of suspension.

The cell is of circular design, and its cross-section is shown in Fig. 1. The electrode diameter is much larger than the diameter of the sample in order to eliminate uncontrolled and frequency-dependent stray fields. Since the field in the sample space is homogeneous, stray field contributions near the edge of the electrodes do not vary with the load and cancel out if differences with loaded and empty cell are obtained. Polystyrene rings with different bore diameters permitted adjustment of the sample capacitance to the bridge range. Dielectric constants and conductivity values were obtained from total observed sample capacitance C_p and resistance R_p in the following steps:

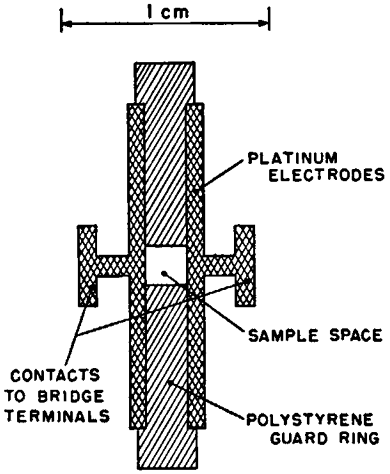

FIGURE 1

1. The readings R_p and C_p are corrected for a series inductance L which is in part caused by the cell and in part by the bridge terminals:

$$R_S = R_p\left[1 + (\omega^2 LC_p)^2 + \left(\frac{\omega L}{R_p}\right)^2\right] \tag{2}$$

$$C_S = \frac{C_p(1 + \omega^2 LC_p) + \frac{L}{R_p^2}}{(1 + \omega^2 LC_p)^2 + \left(\frac{\omega L}{R_p}\right)^2} \tag{3}$$

   R_S and C_S are the true sample resistance and capacitance, ω the angular frequency and L the inductance. The value of L is determined by measurements with several solutions of electrolytes and found to be 8.2×10^{-9} henry.

2. Dielectric constant ϵ and conductivity κ in mho/cm are calculated from sample resistance R_S and capacitance C_S by use of the following equations

$$\epsilon = 18C_S - 37 \tag{4}$$

$$\kappa = 1.59/R_S \tag{5}$$

   where C_S is measured in $\mu\mu$farads and R_S in ohms. The cell constants were determined experimentally. The constant of 37 in equation (4) reflects a shunt capacitance effect. It is largely due to the guard ring arrangement which is used to establish a homogeneous electrical field in the sample.

ANALYSIS OF DATA

Cells, which are surrounded by a membrane, display frequency-dependent capacitance and conductivity values as expressed by the following relaxation equations involving often only one time constant T:

$$\epsilon = \epsilon_\infty + \frac{\epsilon_0 - \epsilon_\infty}{1 + (\omega T)^2} \tag{6}$$

$$\kappa = \kappa_0 + (\kappa_\infty - \kappa_0)\frac{(\omega T)^2}{1 + (\omega T)^2} \tag{7}$$

The subscripts 0 and ∞ indicate limits of ϵ and κ approached at low and high frequencies. T is the time constant characteristic of the relaxation effect expressed by the equations. The values of ϵ_0, ϵ_∞, and κ_0, κ_∞ are interrelated through

$$(\epsilon_0 - \epsilon_\infty)\frac{\epsilon_r}{T} = (\kappa_\infty - \kappa_0) \tag{8}$$

where $\epsilon_r = 8.84 \times 10^{-14}$ farads/cm is the dielectric constant of free space. The determination of the limit values κ_0 and κ_∞ is greatly aided by use of Cole-Cole plots in the admittance plane, (Cole, 1928). This is demonstrated in Fig. 2. Simul-

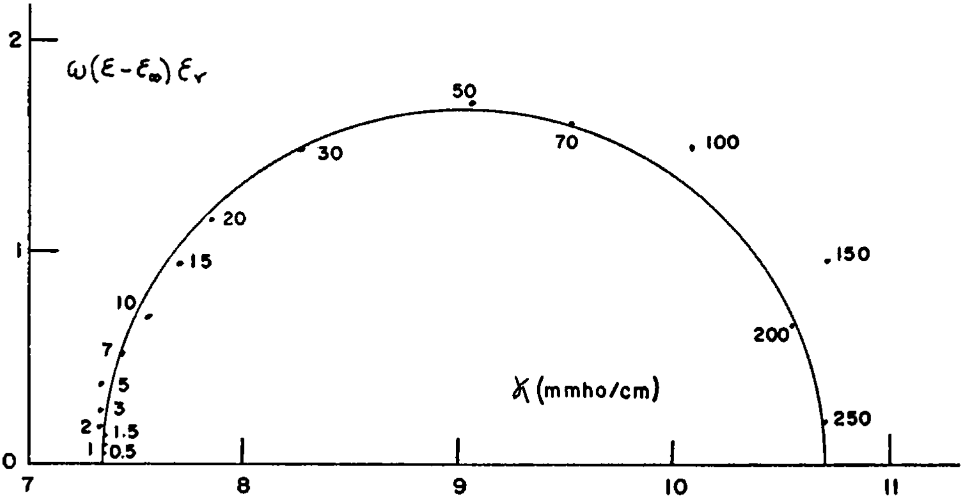

FIGURE 2

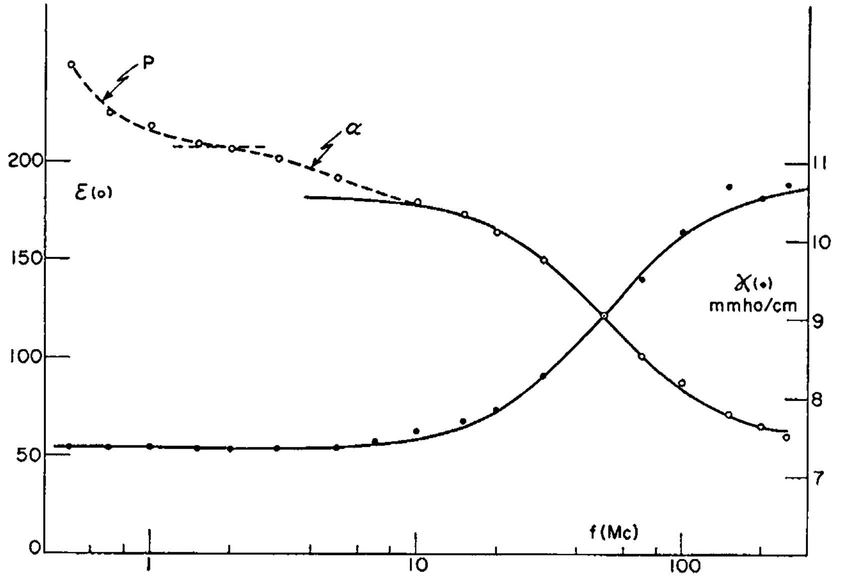

FIGURE 3

taneous use of the Cole-Cole plot, appropriate plots of the dispersion equations (6) and (7) demonstrated in Fig. 3 and use of equation (8) lead to a fairly precise knowledge of ϵ_0, ϵ_∞, κ_0, κ_∞, and the time constant T. Final uncertainty in these data is usually much less than 10 per cent.

The quantities ϵ_0, ϵ_∞, κ_0, κ_∞, and T are related to the following structural parameters:

κ_i, κ_a = conductivity of the interior and suspending medium, respectively;
ϵ_i, ϵ_a = dielectric constant of interior and suspending medium;
p = volume fraction occupied by cells;
d = membrane thickness;
R = cell radius;
C_M = capacity of membrane per unit area.

The pertinent equations are derived in the appendix and permit the calculation of the quantities C_M and κ_i from observed ϵ and κ values.

Assumptions underlying these formulas are:

1. The cellular structure of interest has a spherical shape. This assumption is well fulfilled in the present case. In the case of non-spherical shape or pronounced variability of size, the single time constant must be replaced by a distribution function of time constants. This yields a more flattened type of frequency dependence (Cole and Cole, 1941). Comparison of experimental data with the curve calculated on the assumption of only one time constant (solid line in Fig. 2) is however found to be excellent in the present case.[1]
2. The media inside and outside the cell have predominantly but not necessarily purely resistive and frequency-independent properties. This assumption is well fulfilled at frequencies up to 100 Mc, as may be seen from a comparison of resistive and reactive currents based on the data presented in Table I.
3. The membrane capacitance is nearly frequency independent. Data with many other cellular structures shows this to be a fairly good assumption over most of the frequency range. However, at lower frequencies the assumption is violated and an additional "α-dispersion" appears (Schwan 1957). This is caused at least in good part by relaxation effects associated with counter-ions surrounding the cellular structure.[2]

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[1] Only very pronounced variation in size yields a noticeable flattening of the dispersion curves. For example the usual variability of mitochondrial size is responsible for only minor effects on the dispersion curve of mitochondria (Pauly, Packer, and Schwan, 1960). Deviation from spherical shape has a more pronounced effect. Thus an ellipsoidal shape requires at least two time constants, their ratio being nearly equal to the axis ratio of the ellipsoid. This was clearly evident in work with *E. coli*, (Fricke, Schwan, Li, and Bryson, 1956). The agreement of experimental data with a simple dispersion curve of the type presented in the equations (6) and (7) is therefore typical of spherical cells. However, it does not preclude the possibility of some variability in cellular size.

[2] A detailed theory of this phenomenon has been developed by Schwarz (1962). The slight, but noticeable increase of ϵ above the ϵ_0 indicated by the low frequency level of the solid curve in Fig. 2 is in good part due to this effect. The effect has no bearing on the radio-frequency data of primary interest here.

TABLE I

PPLO DATA

	I	II	III
P	0.49	0.38	0.25
κ_a (mmho/cm)	15.1	13.7	5.35
κ_0 (mmho/cm)	5.8	7.35	3.7
κ_∞ (mmho/cm)	9.8	10.7	5.2
ϵ_0	207	182	168
ϵ_∞	57	62	63
f_0 (Mcps) $= 1/2\pi T$	48	50	25
$RC_M\ [1 \times 3d/R]$(μf/cm)	22×10^{-6}	22×10^{-6}	24×10^{-6}
C_M (μf/cm^2)	1.3	1.3	1.4
κ_i (mmho/cm)(from f_0)	11	12	8
κ_i (mmho/cm)(from κ_∞)	9	12	8

Our approach has been to first calculate C_M from the dielectric constant ϵ_0 (equations 10 and 19, Appendix). Next the internal conductivity κ_i was obtained from the equations (17) and (9) from the time constant T, using the value of C_M obtained from ϵ_0. Finally κ_i was determined from κ_∞ (equations 24, 23, and 9) and found to agree with the value obtained from T, thus demonstrating the internal consistency of the data and the model used in the derivation of the equations in the Appendix.

EXPERIMENTAL RESULTS

Fig. 3 presents a typical set of dispersion curves obtained with PPLO washed in normal nutrient solution. The slight increase in ϵ at very low frequencies, marked P, is due to electrode polarization. This became obvious from measurements with electrolytes of similar ionic strength. The solid curves are calculated from the dispersion equations (6) and (7), using appropriately determined parameters ϵ_0, ϵ_∞, κ_0, κ_∞, and T. The agreement with the experimental data is quite satisfactory. For example the largest deviation in conductance data from the theoretical curve is 3 per cent and observed at very high frequencies, where the resolution of the bridge decreases. Data obtained with three preparations, two of differing ionic strength of the suspending medium, are given in Table I. The over-all consistency of the data is apparent from the agreement of the internal conductance data calculated from ϵ_0 and T and from κ_∞. A 20 per cent deviation in case I reflects in large measure the sensitivity of κ_i from κ_∞. For example a 3 per cent error in κ_∞ causes a 10 per cent error in κ_i.

R has a value of 0.125 μ. A value of 0.1 was assumed for d/R. This corresponds to a membrane thickness of 125 A which is well within the range of values usually quoted for other membranes of similar capacitance. The final data do not depend critically on the assumed d/R-ratio. Hence the consistency achieved with $d/R = 0.1$ does not necessarily reflect a determination of d/R.

DISCUSSION

The existence of cell membranes is highly probable from the high dielectric constants observed at low frequencies. The membrane capacitance is calculated to be 1.3 μf/cm^2 in cases I and II and 1.4 μf/cm^2 in case III. These values are considered identical in view of our accuracy estimate of about 10 per cent. The membrane capacitance values are comparable with data found for other membranes (Cole, 1942; Cole and Curtis, 1950; Schwan and Cole, 1960). This fact and equation 1 suggest a membrane thickness similar to that observed for other cellular structures in agreement with our pertinent result. The dielectric constant of the membrane ϵ_M is determined by $C_M = 1.3$ μf/cm^2 and $d \sim 125$ A. Its value is about 18.[3]

The conductivity of the internal phase is lower than that of the external medium in cases I and II, *i.e.* a suspending medium of normal tonicity.

The lower conductivity of the internal phase reflects primarily the presence of macromolecular structures (Schwan, 1957). They effectively reduce the volume available for ionic conduction and further reduce ionic conduction due to their ion-binding properties. However in case III, where the ionic strength of the external medium was reduced threefold, the internal conductivity responded only with a small corresponding decrease. Thus the internal ionic strength of the microorganism exhibits remarkable independence from the external medium. A similar behavior has been observed for subcellular fractions (Pauly and Packer, 1960).

The striking feature of these studies is the similarity between the electrical characteristics of PPLO membranes and those of other cells.

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[3] Membrane structures often consist of a lipid layer of about 30 A or 40 A, which is covered by protein layers of similar thickness. Thus the over-all capacitance of a membrane may be written

$$\frac{1}{C_M} = \frac{d}{\epsilon_M} = \frac{2d_p}{\epsilon_p} + \frac{d_L}{\epsilon_L}\,; \qquad d = 2d_p + d_L \tag{9}$$

where d_p and d_L are the thickness of the lipid and protein layers and ϵ_p, ϵ_L their respective dielectric constants. The protein layers have probably a fairly high dielectric constant of about 20 or 30 as suggested by determinations of the effective dielectric constant of hydrated proteins (Schwan, 1957). The lipid layer on the other hand has probably a dielectric constant of only 5 or 6 since it has no molecular components which strongly polarize in an electrical field. The proteins are contributing therefore little, perhaps only 30 per cent to the over-all capacitance value. Thus the observation of a capacitance of about 1μf/cm^2 and a membrane dielectric constant of 18 may reflect in good part the presence of a lipid layer of about 40 A thickness, while the over-all membrane may be threefold thicker.

APPENDIX

1. The physical model upon which the analysis of the electrical properties of suspensions of spherical cells is based is shown in Fig. 4. Each cell is approximated by a concentric arrangement of two spheres which contain the cell membrane. In the most general case all three phases involved, cell interior and exterior and cell membrane, are characterized by complex conductivities $K = \kappa + j\omega\epsilon_r\epsilon$, including both dielectric constants ϵ and conductivities κ.

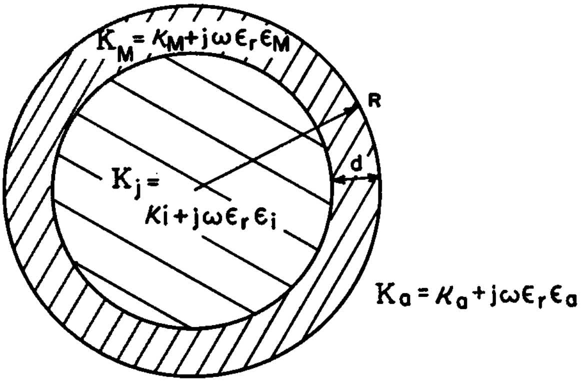

FIGURE 4

The conductivity of a suspension of stratified spheres was determined first by Maxwell (Maxwell, 1873).[4] His equation can be shown to apply also in the case of complex conductivities and then reads

$$K = \frac{(1 + 2p)K_p + 2(1 - p)K_a}{(1 - p)K_p + (2 + p)K_a} K_a \tag{1}$$

where K_p is the "equivalent homogeneous complex conductivity" of a homogeneous particle which behaves in an electrical field exactly as the stratified model and where

$$\frac{K_p - K_M}{K_p + 2K_M} = \left(1 - \frac{d}{R}\right)^3 \frac{K_i - K_M}{K_i + 2K_M} \tag{2}$$

p is the volume fraction taken by the cells.

All other symbols are defined by Fig. 4. Previous discussions of the equations (1) and (2) have been based on the assumption that d is vanishingly small in comparison with the cell radius R. We will drop this assumption, but otherwise follow essentially a procedure outlined by Cole (Cole, 1928).

2. Introduction of the quantity

$$\Delta = d\left[1 - \frac{d}{R} + \frac{1}{3}\left(\frac{d}{R}\right)^2\right] \tag{3}$$

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[4] Maxwell's equation is based on a specific application of the principle of superposition which has been subject to criticism. However, his result merges with more modern approaches for small volume fractions p. Experimental evidence suggests that Maxwell's formula applies quite well to volume fractions of up to 0.5 and sometimes even more.

permits replacement of $(1 - d/R)^3$ by $1 - 3\Delta/R$. Thus, solving equation (2) for K_p

$$K_p = K_i \frac{1 - 2\frac{\Delta}{R} + 2\frac{\Delta}{R}\frac{K_M}{K_i}}{1 - \frac{\Delta}{R} + \frac{\Delta}{R}\frac{K_i}{K_M}} \tag{4}$$

By definition, $d < R$. Hence, from equation (3), $\Delta/R < 0.33$. Furthermore it is safe to *assume* that for the frequency range of present interest

$$|K_M| < \tfrac{1}{3}\,|K_i| \tag{5}$$

For example, for a membrane dielectric constant ϵ_M of 15 and an internal cell conductivity κ_i of 10 mmho/cm, *i.e.* values typical for PPLO, the frequency must be larger than 400 Mc to violate the assumption (5). Hence,

$$\frac{\Delta}{R}\left|\frac{K_M}{K_i}\right| < 0.1 \tag{6}$$

In the case of the PPLO the term $(\Delta/R)|(K_M/K_i)|$ will be smaller than 0.01 for frequencies below 100 Mc. This justifies the neglect of this term in the numerator of equation (4). Consequently

$$K_p = K_i \frac{1 - 2\frac{\Delta}{R}}{1 - \frac{\Delta}{R} + \frac{\Delta}{R}\frac{K_i}{K_M}} \tag{7}$$

or

$$\frac{K_i'}{K_p} = 1 + \frac{K_i'}{K_M'}\frac{d}{R} \tag{8}$$

with

$$K_i' = \frac{1 - 2\frac{\Delta}{R}}{1 - \frac{\Delta}{R}} K_i; \qquad K_i \sim K_i'\left(1 + \frac{d}{R}\right) \tag{9}$$

$$K_M' = \frac{K_M}{1 - 2\frac{\Delta}{R}}\frac{d}{\Delta}; \qquad C_M \sim C_M'\left(1 - 3\frac{d}{R}\right) \tag{10}$$

where $C_M = \epsilon_M\epsilon_r/d$ is the membrane capacitance per surface unit. The equations (8) to (10) state that it is permissable to use equations which apply in the special case where d vanishes in comparison with R even though this condition may not be fulfilled. However, if d is not very small compared to R the then-observed quantities K_i' and C_M' must be corrected as indicated in order to obtain K_i and C_M.

We can further simplify the expression for K_p by assuming that the membrane conductivity κ_M can be neglected. This assumption is permissible since membrane conductance values must be much larger than those reported in the literature in order to contribute noticeably to K_M. The assumption is also supported in the case of the present study by the ability of the model discussed to quantitatively explain the experimental data. With $\kappa_M = 0$ equation (8) reduces to

$$K_p = \frac{K_i' \cdot j\omega RC_M'}{K_i' + j\omega RC_M'} \tag{11}$$

3. We will now introduce the expression for the equivalent homogeneous conductivity (11) into the equation (1). This yields

$$\frac{K}{K_a} = \frac{j\omega A - R\omega^2 C_M'\epsilon_r[(1 + 2p)\epsilon_i' + 2(1 - p)\epsilon_a] + 2(1 - p)[\kappa_a\kappa_i' - \omega^2\epsilon_a\epsilon_i'\epsilon_r^2]}{j\omega B - R\omega^2 C_M'\epsilon_r[(1 - p)\epsilon_i' + (2 + p)\epsilon_a] + (2 + p)[\kappa_a\kappa_i' - \omega^2\epsilon_a\epsilon_i'\epsilon_r^2]} \tag{12}$$

with

$$A = RC_M'[(1 + 2p)\kappa_i' + 2(1 - p)\kappa_a] + 2\epsilon_r(1 - p)(\kappa_a\epsilon_i' + \kappa_i'\epsilon_a) \tag{13}$$

$$B = RC_M'[(1 - p)\kappa_i' + (2 + p)\kappa_a] + \epsilon_r(2 + p)(\kappa_a\epsilon_i' + \kappa_i'\epsilon_a) \tag{14}$$

Now we make the further *assumptions*

$$\kappa_a \gg \omega\epsilon_a\epsilon_r; \qquad \kappa_i' \gg \omega\epsilon_i'\epsilon_r; \qquad R\omega C_M \ll \kappa_a,\ \kappa_i \tag{15}$$

The applicability of these assumptions up to frequencies in excess of 100 Mc is supported by the values given in Table I. With the assumptions (15) equation (12) reduces to

$$\frac{K}{K_a} = \frac{1 - p}{1 + \frac{p}{2}} \frac{1 + j\omega\left[RC_M'\left(\frac{1}{\kappa_i'} + \frac{1 + 2p}{1 - p}\frac{1}{2\kappa_a}\right) + \epsilon_r\left(\frac{\epsilon_i'}{\kappa_i'} + \frac{\epsilon_a}{\kappa_a}\right)\right]}{1 + j\omega\left[RC_M'\left(\frac{1}{\kappa_i'} + \frac{1 - p}{1 + p/2}\frac{1}{2\kappa_a}\right) + \epsilon_r\left(\frac{\epsilon_i'}{\kappa_i'} + \frac{\epsilon_a}{\kappa_a}\right)\right]} \tag{16}$$

This expression in turn reduces to the dispersion equations (6) and (7) with the parameters

$$T = RC_M'\left[\frac{1}{\kappa_i'} + \frac{1 - p}{1 + \frac{p}{2}}\frac{1}{2\kappa_a}\right] + \epsilon_r\left(\frac{\epsilon_i'}{\kappa_i'} + \frac{\epsilon_a}{\kappa_a}\right) \tag{17}$$

$$\kappa_0 = \kappa_a \frac{1 - p}{1 + \frac{p}{2}} \tag{18}$$

$$\epsilon_0 = \epsilon_a \frac{1 - p}{1 + \frac{p}{2}} + RC_M' \frac{9}{4\epsilon_r} \frac{p}{\left(1 + \frac{p}{2}\right)^2} \tag{19}$$

$$\epsilon_\infty = \epsilon_a \frac{1 - p}{1 + \frac{p}{2}} \tag{20}$$

κ_∞ may be obtained from above values with the aid of equation (8) in the text. The assumptions (15) are somewhat more questionable than those made previously since they are not necessarily any more fulfilled at frequencies above 100 Mc. Hence at frequencies in excess of 100 Mc experimental curves may deviate from the simple dispersion curves characterized by one time constant. This is due to the appearance of the ω^2 terms which are missing in the simple expression (16) but not in the more complete form (14). Hence, extrapolation of the simple dispersion curves observed below 100 Mc gives results which would pertain if the ω^2 terms would not exist; *i.e.* if the assumptions were fulfilled. The equation (20) for ϵ_∞ particularly reflects the assumptions made, since it does not reflect the existence of dielectric constants associated with membrane and cell interior. A detailed discussion of this problem can be based on the more general treatment given before (Pauly and Schwan, 1959). It reveals that the error of ϵ_∞ in equation (20) is fairly small, perhaps some dielectric units. The validity of the expressions for T, ϵ_0 and κ_0 is of course even less affected by the assumptions (15), since ϵ_0 and κ_0 are obtained at very low frequencies and T is not critically affected by some variation in ϵ_∞.

4. A formula which relates ϵ_∞ to the cell's dielectric properties is obtained directly from equation (1). This equation reduces for very high frequencies to

$$\epsilon = \frac{(1 + 2p)\epsilon_p + 2(1 - p)\epsilon_a}{(1 - p)\epsilon_p + (2 + p)\epsilon_a} \cdot \epsilon_a \tag{21}$$

where the dielectric constant of the cell ϵ_p is related to the dielectric constant of the interior ϵ_i by equation (2) which reads now

$$\frac{\epsilon_p - \epsilon_M}{\epsilon_p + 2\epsilon_M} = \left(1 - \frac{d}{R}\right)^3 \frac{\epsilon_i - \epsilon_M}{\epsilon_i + 2\epsilon_M} \tag{22}$$

This simplifies further if we consider the fact that the dielectric constant of the membrane ϵ_M is much smaller than both ϵ_i and ϵ_p. The equations (21) and (22) have been used to calculate ϵ_i —values. These values are for the PPLO near 50, which is the expected range (Schwan, 1957) on the basis of the composition of the PPLO (Morowitz *et al.*, 1962). Slight errors of this, reflecting some uncertainty about ϵ_M, are of no consequence since small errors in ϵ_i are further attenuated in the determination of κ_i from equation (17) due to the fact that the term which contains ϵ_i has only a minor effect on κ_i.

5. In an attempt to directly obtain κ_i from κ_∞ we rearrange equation (8) and solve for κ_p. Thus

$$\frac{\kappa_p}{\kappa_i'} = \frac{RC_M' - \epsilon_p\, \epsilon_r}{RC_M' + \epsilon_i'\, \epsilon_r} \tag{23}$$

κ_p is obtained at high frequencies in a close approximation[5] from equation (1)

$$\kappa_\infty = \frac{(1 + 2p)\kappa_p + 2(1 - p)\kappa_a}{(1 - p)\kappa_p + (2 + p)\kappa_a}\, \kappa_a \tag{24}$$

Equations (23) and (24) permit a direct determination of κ_i and κ_∞. Pertinent results are given in Table I and in good agreement with the values obtained from T.

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[5] The simultaneous validity of the equations (21) and (24) is related to the fact that the overall frequency response of the model shown in Fig. 4 is characterized by two time constants. However the time constant which determines the behavior at frequencies near to and in excess of 100 Mc is simply that of a sphere without shell; *i.e.* that of the cell without membrane. This dispersion effect is characterized by a low frequency conductivity given in equation (24) and a high frequency dielectric constant as stated in (21). It is of small magnitude so that it can be neglected from a practical point of view. Hence the κ and ϵ values given in the expressions (21) and (24) are good approximations over the range of frequencies covered by this insignificant relaxation effect. For more detailed information about this see Pauly and Schwan, 1959 and Schwan, 1957, p. 199.

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