Electrical Properties of Mitochondrial Membranes*

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(Received for publication, July 4, 1959)

ABSTRACT

The electrical capacity of the membrane of rat liver mitochondria is 0.5 to 0.6 μf./cm^2. This membrane capacity is obtained from the analysis of the frequency dependence of the admittance of a suspension of swollen mitochondria.

In potassium chloride media the mitochondrial membrane capacity does not depend on the ion concentration.

The internal conductance of the mitochondria was approximately one-half that of the external medium; the same applies if the mitochondria are equilibrated in a medium with a 10-fold difference in potassium chloride concentration. Hence the swollen mitochondria investigated here appear to be able to adjust their internal ion concentration in proportion with that of the external phase.

The similarity of the membrane capacity of isolated mitochondria with the range of values known for other membranes suggests a common molecular structure.

The analysis of experimental data suggests an anisotropic electrical behavior of the interior of mitochondria. This anisotropy is readily explained by the existence of internal membranes.

INTRODUCTION

In recent years there have been a number of reports that isolated mitochondria exhibit osmotic properties (Claude, 1946; Tedeschi and Harris, 1955, 1958; Jackson and Page, 1956; Recknagel and Malamed, 1958). Electron microscopical evidence (Palade, 1953), permeability studies (de Duve and Berthet, 1954), in addition to osmotic properties suggest that mitochondria are surrounded by a membrane. Historically, similar evidence was invoked to prove the existence and to show the properties of the cell membrane. A comparison of the electrical properties of mitochondrial and cell membranes may help to clarify their structural relationship.

Ruhenstroth-Bauer and Zeininger (1956) reported that mitochondria are surrounded by a membrane of relatively high electrical resistance as shown by measurements of the frequency dependence of the conductivity of a mitochondrial suspension, but were unable to state actual electrical membrane properties. In this article it will be shown that the analogy between the cell and mitochondrial membrane goes further, as ascertained by membrane capacity measurements of rat liver mitochondria.

A complete theory of the frequency dependence of the electrical admittance of a suspension of shelled spheres is available (Pauly and Schwan, 1959). Since mitochondria isolated from animal tissues assume a spherical shape *in vitro*, it is possible to analyze the measured electrical dispersion curves, taking advantage of a well founded theory based on equations given originally by Maxwell (1892). An assumption is made that mitochondrial size does not significantly change the electrical properties of the outer membrane. The justification for this assumption will be presented in a subsequent article (Pauly and Packer, 1960).

Preparation

Rat liver mitochondria were prepared in 0.25 M sucrose according to the method recommended by

* This work was supported in part by the United States Office of Naval Research (NONR-551 (05)) and in part by the United States Public Health Service (USPH-1253 (C6)).

‡ E. R. Johnson Foundation for Medical Physics, University of Pennsylvania, Philadelphia.

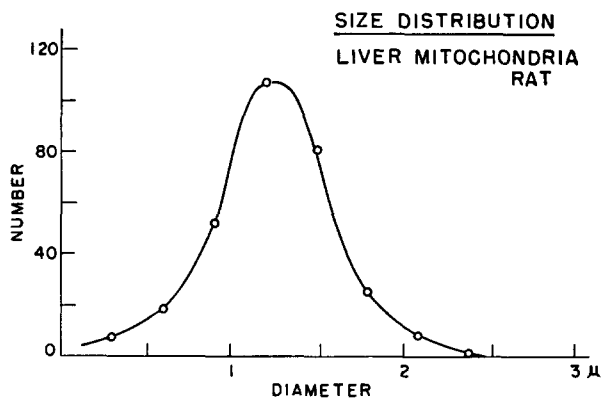

FIG. 1. Size distribution of rat liver mitochondria after swelling in 0.001 M KCl solution and equilibration in 0.012 M KCl solution (Experiment A) or 0.13 M KCl solution (Experiment B).

Schneider and Hogeboom with the exception that the washing procedure was carried out four times. In this state the mitochondria were small and shrunken. In the phase contrast microscope they appeared dark, indicating a high concentration of internal substances possessing a relatively high refractive index. The average diameter in this state was 0.5 to 0.7 μ.

In order to transform the mitochondria into a spherical shape, the suspension was diluted 10-fold in 0.001 M KCl. The suspension was then incubated for 2 hours at room temperature to allow for equilibration of the internal and external phase. Following this, the suspension was centrifuged, washed, and equilibrated in the same manner two additional times in order to insure that equilibration was complete. The sediment was suspended in the final KCl concentration employed for the dielectric measurement.

A knowledge of the size distribution is necessary to interpret properly the experimental admittance data. This was done with the phase contrast microscope (Leitz dialux with the Heine condenser, phase contrast oil immersion objective, periplane ocular × 25, and a calibrated ocular micrometer).

The result is shown in Fig. 1. The distribution of the diameter is nearly symmetrical with the most probable diameter near 1.2 μ. The size distributions for experiment A and B (Fig. 3) were found to be identical. This is not surprising considering the fact that in both cases internal and external media were equilibrated.

The conventional techniques employed for the isolation of liver mitochondria used in the present experiments are known to yield preparations largely free of contamination from other cellular structures. These conclusions have been based on microscopic examination of preparations and assay of such enzymatic activities as succinoxidase and cytochrome oxidase which are found exclusively in mitochondria (Siekevitz and Watson, 1956). Although phase contrast microscopy of liver mitochondria after impedance measurements showed no evidence of damage to the mitochondrial structure, it seemed desirable as a control to disrupt mitochondria and then examine their electrical properties. Mitochondrial membrane fragments were prepared from intact liver mitochondria by disruption with digitonin according to the method of Devlin and Lehninger (1958). Phase contrast microscopy, and also electron microscopy (*cf.* Siekevitz and Watson, 1957) show that fragment preparations are completely free of whole mitochondria. Impedance measurements showed that the electrical capacity of whole mitochondria is not retained by the fragments even when the latter were tested at volume concentrations as high as 50 per cent. These findings lend additional support to the interpretation of the impedance measurements on intact mitochondria reported below.

Dielectric Measurements

1. Bridge.—The admittance of the sample was measured with the "RX-Meter type 250-A" of the Boonton Radio Corporation, Boonton, New Jersey. It is designed to measure the equivalent parallel resistance, R_p, in ohms, and the parallel capacitance, C_p, in $\mu\mu$f., of the sample, between 5×10^5 and 2.5×10^8 C.P.S. The instrument consists of a Schering bridge circuit together with its associated oscillator, amplifier, null detector, and power supply. Bridge balance was obtained by means of two calibrated air capacitors, which indicate parallel resistance and parallel capacitance, respectively.

2. Cell.—In order to avoid a frequency dependent stray field, the cell, shown in Fig. 2, was constructed. The cell is essentially a parallel plate condenser with 2 platinum electrodes, which were platinized to minimize electrode polarization effects. The sample was placed in the cylindrical lumen in the 2 mm. thick polystyrene ring. Rings with different bore diameters were used to adjust the capacity of the sample to the range of the bridge for optimal resolution. Since the field in the sample space and the part of the poly-

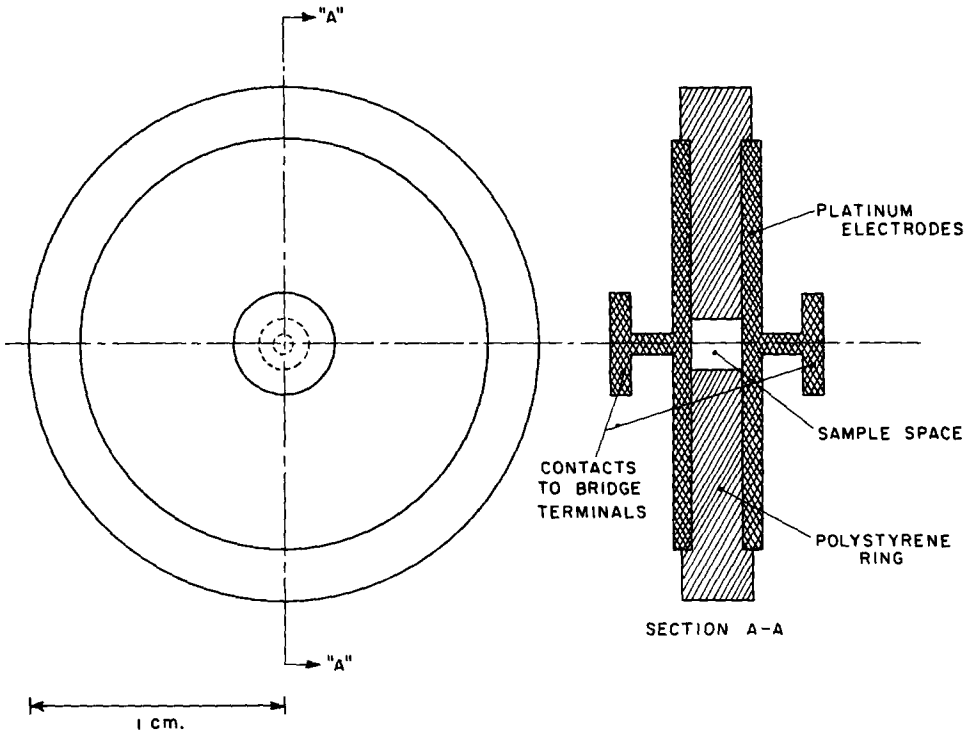

FIG. 2. Cell for dielectric measurement. For description see text.

styrene ring bordering on this space were homogeneous, stray field components near the edges of the plates do not depend on the sample load, and are, therefore, frequency independent. Hence a linear relationship between sample dielectric constant and total capacitance was obtained. It was found convenient to connect the cell to the bridge terminal by a mercury contact. The contact resistance was found to be negligible.

The dielectric constant ϵ of the sample was obtained from

$$\epsilon = 1 + (\epsilon_{Aq} - 1)\frac{C_S - C_{AIR}}{C_{Aq} - C_{AIR}} \qquad (1)$$

in which

ϵ_{Aq} = Dielectric constant (DK) of water, obtained from tables,

C_S = Capacity of the cell with the sample, after correction for the series inductance L, due to leads to the sample cell,

C_{Aq} = Capacity of the cell, filled with water and corrected for L,

C_{AIR} = Capacity of the empty cell, corrected for L.

The cell constant for the conductivity was obtained by calibration with a standard 0.1 normal KCl solution. There was good agreement between the cell constant obtained by calibration and that calculated from the dimension of the cell. Similarly, the cell constant for the dielectric constant was obtained by calibration with water, as indicated in equation (1) and checked well with that for the conductivity.

3. Corrections.—An frequencies in excess of 50 Mc., the series inductance L of the connected cell and bridge terminals causes considerable error in sample conductance and capacitance, especially in highly conductive media. The correction was made by means of the equations

$$R = R_p\left[(1 + \omega^2 L C_p)^2 + \left(\frac{\omega L}{R_p}\right)^2\right] \qquad (2)$$

$$C = \frac{C_p(1 + \omega^2 L C_p) + \dfrac{L}{R_p^2}}{(1 + \omega^2 L C_p)^2 + \left(\dfrac{\omega L}{R_p}\right)^2}, \qquad (3)$$

in which R = resistance in ohm,

C = capacitance in farad,

R_p = measured equivalent parallel resistance in ohm,

C_p = measured equivalent parallel capacity in farad,

L = series inductance, 8.2×10^{-9} henry,

$\omega = 2\pi f$,

f = frequency in C.P.S.

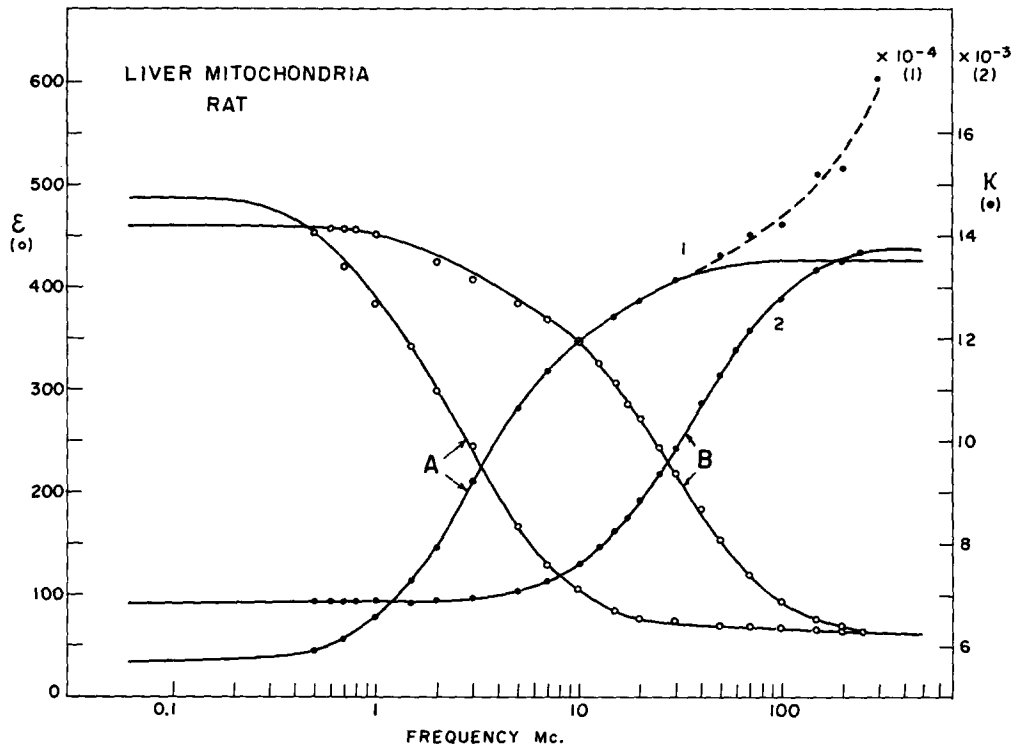

FIG. 3. β-dispersion of swollen rat liver mitochondria.

A. Suspension in 0.012 M KCl solution.
B. Suspension in 0.13 M KCl solution.
Temperature 25°C.

TABLE I

Rat Liver Mitochondria

Summary of the data and final values for the membrane capacity and internal conductivity. For explanation see text.

Experiment	KCl	κ_0	κ_∞	κ_a	κ_i	$\frac{\kappa_i}{\kappa_a}$	P	ϵ_0	ϵ_∞	$f_0 = (f_{0\epsilon} f_{0\kappa})^{1/2}$	C_M from ϵ_0	C_M from f_0
	mM.	*m mho/cm.*	*m mho/cm.*	*m mho/cm.*	*m mho/cm.*					*Mc.*	*μf./cm.²*	*μf./cm.²*
A	12	0.57	1.35	1.84	1.07	0.58	0.60	480	64	2.8	0.50	0.61
B	130	6.8	13.7	18.7	10.0	0.54	0.53	440	65	27	0.49	0.62
Dilution series	92	13.2 -3.9	/	13.2	/	/	0–0.51	76–430	/	/	0.51	/

4. β-Dispersion of the Mitochondria Suspension.[1]—The frequency dependence of mitochondria suspensions at two KCl concentrations, differing by a factor of about 10 is shown in Fig. 3. The data, together with the results of the analysis of the dispersion curves are summarized in Table I.

[1] The "structural" relaxation effect of interest here is of the "β-type" following the nomenclature introduced by Schwan (1957, 1959). Other dispersion phenomena at different frequency ranges and of different origin are observed in tissues and cell suspensions (Schwan, 1957, 1959).

Analysis of Data

1. Theory.—The dispersion curves were analyzed by application of an appropriate extension of Maxwell-Wagner's theory of inhomogeneous di-

electrics (Maxwell, 1892). The mathematical case, which was fitted to this problem, was that for a suspension of spheres with the DK ϵ_i and specific conductivity κ_i, surrounded by a shell (membrane) with the DK ϵ_s and κ_s suspended in an outside medium with the electrical properties ϵ_a and κ_a, and has been discussed in detail (Pauly and Schwan, 1959). The frequency dependence of a suspension of shelled spheres can be described by a superposition of two Debye-expressions of the form

$$\epsilon = \epsilon_\infty + \frac{\epsilon_0 - \epsilon_\infty}{1 + (\omega T)^2}\,; \qquad \kappa = \kappa_0 + (\kappa_\infty - \kappa_0)\,\frac{(\omega T)^2}{1 + (\omega T)^2} \tag{4}$$

In the special case of a relatively thin shell with a negligible conductance compared to the inside and outside media, the following approximations hold (Fricke, 1924; Cole, 1928, *a*, *b*; Dänzer, 1934, *a*, *b*; Schwan, 1957):

$$T = \frac{1}{2\pi f_0} = R \cdot C_M\,\frac{\kappa_i + 2\,\kappa_a}{2\,\kappa_i \cdot \kappa_a} \tag{5}$$

$$\epsilon_0 \approx \epsilon_a + \frac{9}{4\epsilon_r}\,p \cdot R \cdot C_M \tag{6}$$

$$\epsilon_\infty \approx \epsilon_a\,\frac{(1 + 2p)\,\epsilon_i + 2\,(1 - p)\,\epsilon_a}{(1 - p)\,\epsilon_i + (2 + p)\,\epsilon_a} \approx \epsilon_a\,, \tag{7}$$

$$\kappa_0 \approx \kappa_a\,\frac{1 - p}{1 + \frac{p}{2}} \tag{8}$$

$$\kappa_\infty \approx \kappa_a\,\frac{1 + 2p\,\frac{\kappa_i - \kappa_a}{\kappa_i + 2\kappa_a}}{1 - p\,\frac{\kappa_i - \kappa_a}{\kappa_i + 2\kappa_a}} \tag{9}$$

In equations (4) to (9)

T = relaxation time [sec.],
f_0 = characteristic frequency [C.P.S.],
ϵ_0 = low frequency dielectric constant of the suspension,
ϵ_∞ = high frequency dielectric constant of the suspension,
κ_0 = low frequency conductivity of the suspension [mho/cm.],
κ_∞ = high frequency conductivity of the suspension [mho/cm.],
ϵ_i, ϵ_a = dielectric constant of the interior and the medium, respectively,
κ_i, κ_a = conductivity of the interior and the medium, respectively [mho/cm.],
ϵ_r = $1/4\pi \times 9 \times 10^{11}$ = dielectric constant of vacuum,
p = volume fraction occupied by the spheres,
R = radius of the spheres [cm.],
C_M = capacity of the membrane (shell) [farad/cm.^{2}],
$C_M = \frac{\epsilon_r\,\epsilon_s}{d}$, in which ϵ_s = the DK of the membrane and d the thickness of the membrane in cm.

Equations (4) to (9) describe a relaxation phenomenon with the single relaxation time T. It has been shown (Cole and Cole, 1941) that the plot $\frac{\kappa - \kappa_0}{\omega\epsilon_r} = \epsilon''$ *versus* ϵ in the dielectric plane, and the plot $\omega\epsilon_r \cdot (\epsilon - \epsilon_\infty)$ *versus* κ in the admittance plane yield semicircles with the center on the abscissa. When a spectrum of relaxation times with a Cole-Cole distribution function (Cole and Cole, 1941) occurs, the center of the semicircle will be depressed. If the distribution function of the relaxation time is not a Cole-Cole function, but a statistical (Gauss-, Poisson- or general Bernoulli-distribution) function, a circle with depressed center is a good approximation of the actual curve in the dielectric or admittance plane (Schwan, 1957). For a given distribution the degree of the depression is different in the dielectric and admittance plane for theoretical reasons. This can be seen by comparison of Figs. 4 and 5.

The dielectric plot of Experiment A in Fig. 3 is given in Fig. 4; the corresponding admittance plot is given in Fig. 5. The centers of those circles in these plots, chosen to approximate the results, are depressed. The same result was found when the data of Experiment B in Fig. 3 were treated in this manner (not shown). Therefore the dispersion curves of Fig. 2 can be described by a superposition of Debye-terms, each with a different time constant T. Systematic deviations from the circle at high frequencies are due to additional relaxation effects located in the cell interior (see dashed line in Fig. 3), as will be discussed later. The values for κ_0, κ_∞, ϵ_0, and ϵ_∞, summarized in Table I were obtained from these plots by extrapolation to the abscissa.

The spectrum of time constants can be explained by:

1. A distribution of the radius R of the swollen mitochondria, since T is a function of R (*cf.* equation (5)).

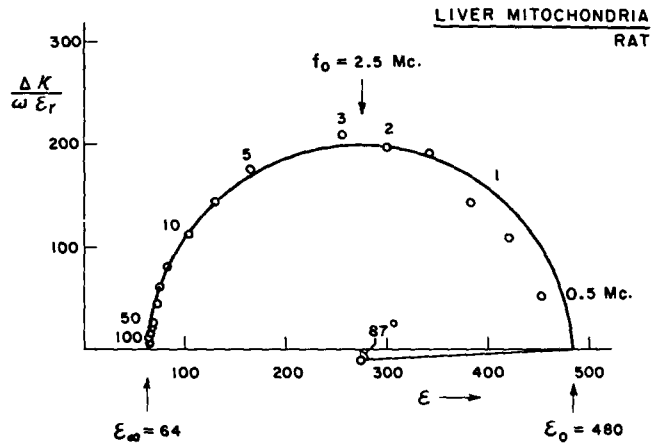

FIG. 4. Plot of $\frac{K - K_0}{\omega \epsilon_r}$ *versus* ϵ of Experiment A in the complex dielectric plane. Frequencies are indicated in Mc. The half-value frequency of the ϵ curve is 2.5 Mc.

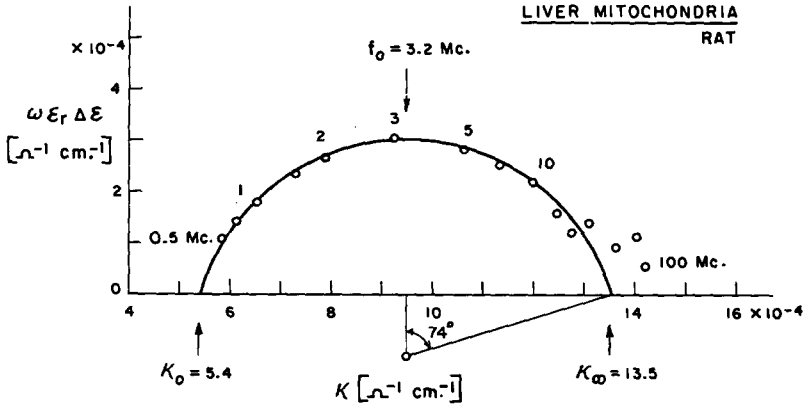

FIG. 5. Plot of $\omega \epsilon_r(\epsilon - \epsilon_\infty)$ *versus* κ of Experiment A in the complex admittance plane. Frequencies are indicated in Mc. The half-value frequency of the κ curve is 3.2 Mc. Note the difference in the half-value frequencies for ϵ and κ, as well as the differences in suppression of the center of the circle in both plots.

2. A distribution of the internal conductance, since T is a function of κ_i (*cf.* equation (5)).

3. A phase angle of the membrane (Cole, 1928 *a*, *b*), or lastly,

4. A combination of the aforementioned possibilities.

2. Calculation of C_M from ϵ_0 and the Size Distribution of the Mitochondria.—From the theoretical point of view, C_M should be obtained most accurately from ϵ_0 by means of equation (6). Since the inside conductance κ_i does not appear in equation (6), a distribution in κ_i cannot effect the results. If we further assume, for the present, a static membrane capacity within the frequency range of the β dispersion, we can anticipate the result of the following estimate to apply to the total investigated frequency range. This assumption seems to be justified at least in some cases, such as erythrocyte membranes (Schwan, 1957) and membranes of *E. coli* (Fricke, Schwan, Li, and Bryson, 1956).

Introducing the superposition principle for the eight points in the size distribution curve given in Fig. 1, equation (6) has been rewritten in the form

$$\epsilon_0 - \epsilon_a = \frac{9}{4\epsilon_r} \cdot C_M \cdot \Sigma_{i=1}^{8} p_i R_i \tag{10}$$

p_i is the corresponding volume fraction of the size group i with the radius R_i. In equation (10) the assumption of a size independent membrane ca-

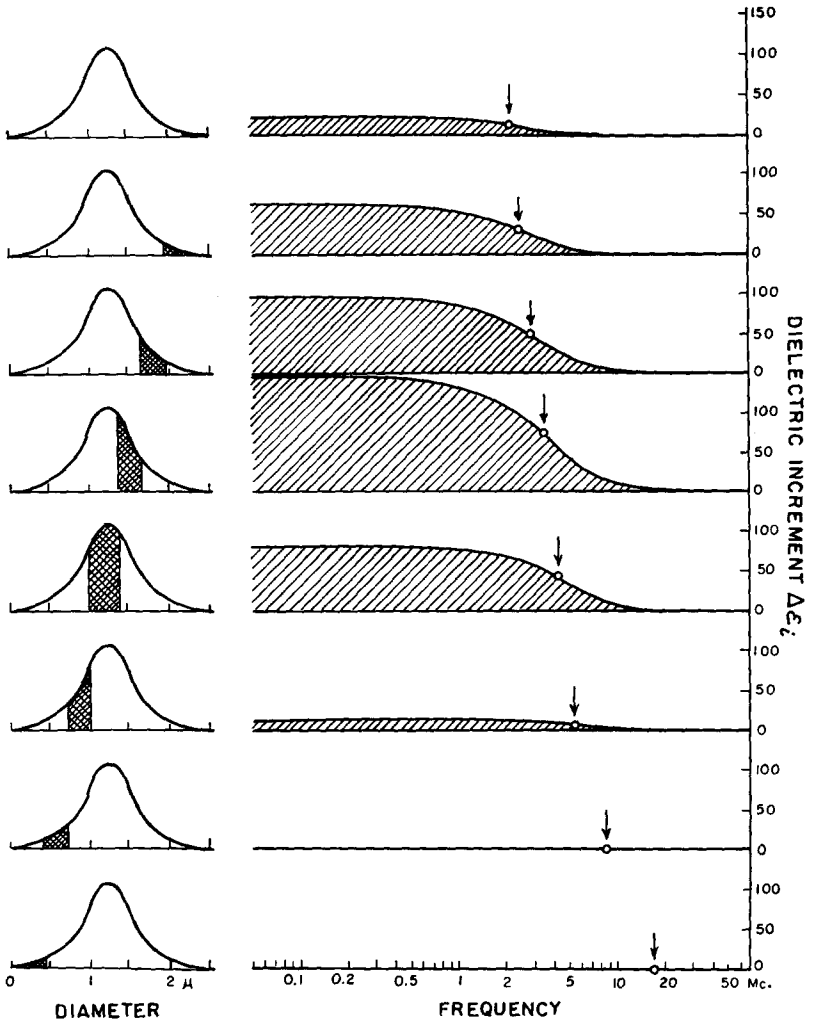

FIG. 6. The graph shows the procedure in calculating the dispersion curve for ϵ for mitochondrial suspensions of different size. The size distribution curve is shown on the left. The dielectric contribution of each of the eight size groups (shaded areas) is plotted on the right of the figure. The largest dielectric contribution arises from the group for which the product $n_i \cdot R_i$ is largest. Note the shift of the characteristic frequency with decreasing diameter to higher frequencies.

pacity is made. The total volume fraction p was calculated from the low frequency conductivity κ_0 and from κ_a by means of equation (8). Attempts to determine the absolute volume concentration by means of packed cell volume determinations (International hematocrit centrifuge, International Equipment Co., Boston) failed on account of incomplete packing and because the degree of packing was dependent upon the outside medium.

With the known values for p, ϵ_0, ϵ_a, and the size distribution, equation (9) can be evaluated.[2] Experiment A yielded for C_M 0.50 μf./cm.^{2}, and experiment B, $C_M = 0.49$ μf./cm.^{2} (Table I). These values lie within the range from 0.5 to 2 μf./cm.^{2}, previously found for erythrocytes (Fricke, 1931), leucocytes (Fricke and Curtis, 1934), bacteria (Fricke, Schwan, Li, and Bryson, 1956), amphibian and sea urchin eggs (Cole and Curtis, 1950).

3. Calculation of C_M from the Time Constant T.—

[2] Equation (5) holds only if $p \ll 1$, but the experimental value was ~0.6; therefore, an experimentally determined correction factor was introduced. The correction factor is discussed later in the text.

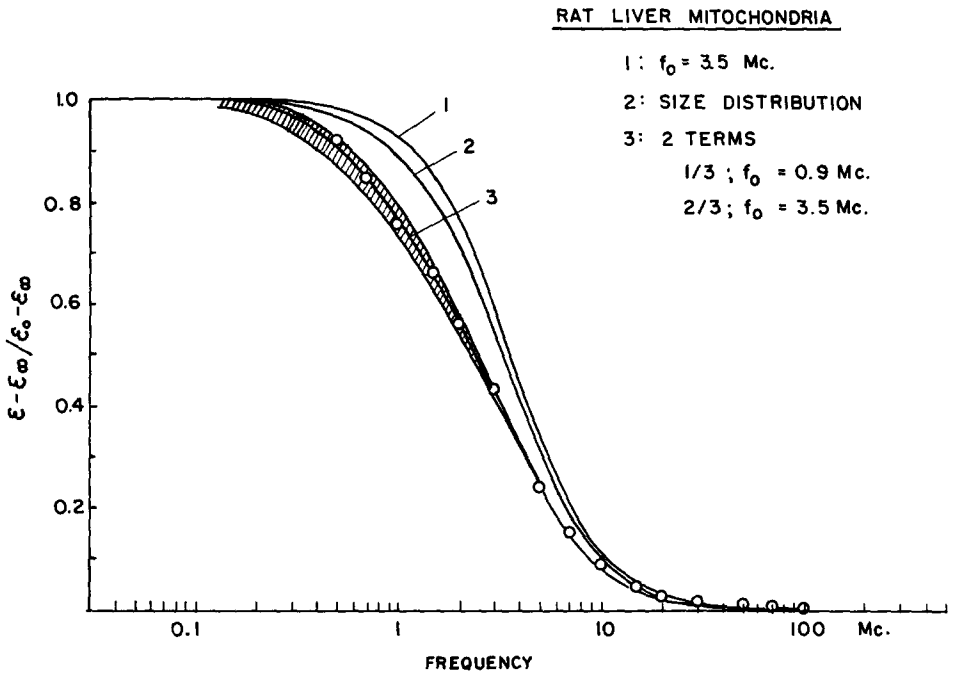

FIG. 7. Comparison of the measured and normalized dielectric constant with the calculated one (Experiment A).

Curve 1. Single relaxation time calculated with $R = 0.75\ \mu$ (size group with the largest contribution to ϵ_0); $\kappa_a = 1.84$ m mho/cm.; $\kappa_i = 1.07$ m mho/cm.; $C_M = 0.50\ \mu$f./cm^2.

Curve 2. Dispersion curve with distribution of time constants as a result of the variability in mitochondrial size. Procedure of calculation see Fig. 6. Values for κ and C_M are those of Curve 1.

Curve 3. Approximation of the experimental curve by an expression with 2 time constants, differing by a factor of 4. The curve connecting the experimental points is calculated by one term with the characteristic frequency $f_0 = 3.5$ Mc. and a normalized low frequency dielectric increment of $\frac{2}{3}$, and second term with $f_0 = 0.9$ Mc. and a normalized low frequency dielectric increment of $\frac{1}{3}$. The shaded area is obtained if the characteristic frequency of the second term varies between $f_0 = 0.7$ Mc. and $f_0 = 1.1$ Mc.

The second and independent method to obtain C_M is by means of T (equation (4)). In the case of a spectrum of time constants, the characteristic frequency for the κ dispersion is not identical with the characteristic frequency of the ϵ dispersion (see Figs. 4 and 5). No general theory for this case is available as yet to relate the average T with C_M. Therefore, we have taken, as a reasonable approximation for f_0, the geometrical average between the two characteristic frequencies. Using κ_i (obtained from equation (9)) and the R average of 0.75 μ for which the contribution to ϵ_0 is maximal (Fig. 6), the C_M values 0.61 and 0.62 μf./cm.^{2} were calculated (Table I). This is in good agreement with the figures obtained by calculation from ϵ_0, considering the assumptions underlying this approach.

4. Distributions of Time Constants due to Variability of Mitochondrial Size.—Suppose the spectrum in T is due to a spectrum in R alone, then agreement between the measured curves and dispersion curves calculated with consideration of the variability in R should exist. The theoretical curve obtained on the basis of only one time constant,

$$\epsilon = \epsilon_\infty + \frac{\epsilon_0 - \epsilon_\infty}{1 + (\omega T)^2}, \tag{11}$$

can be generalized for a distribution of T

$$\epsilon = \epsilon_\infty + \sum_i \frac{\Delta\epsilon_i}{1 + (\omega T_i)^2}, \tag{12}$$

in which $\epsilon_{0i} - \epsilon_{\infty i} = \Delta\epsilon_i$ are the dielectric increments associated with each T_i. $\Delta\epsilon_i$ and T_i are given by equations (5) to (9) and are functions of the frequency independent values p_i, R_i, ϵ_0, ϵ_∞, κ_0, κ_∞, and κ_a. The calculation itself for Experiment A, Fig. 2, is demonstrated in Fig. 6 and is based on a subdivision of the total R distribution curve into eight groups. The T values were obtained with $C_M = 0.5\ \mu$f./cm^2.

At the right of Fig. 6 the ϵ contribution of each of the eight size groups analyzed are plotted together with the characteristic frequency corresponding to the average R within each group, while on the left side the corresponding size group in the size distribution curve is indicated. It can be seen that the small mitochondria contribute much less than the corresponding volume of the larger mitochondria.

The superposition of the eight contributions is shown in Fig. 7, Curve 2, together with the normalized experimental curve. In general, the agreement between the measured and calculated curve is satisfactory from a qualitative point of view. The calculated half-value frequency for ϵ is 3.2 Mc compared to the experimental value 2.4 Mc. Thus it appears that the theory employed for the examination of the mitochondrial system approximates the data.

However, there are significant differences with respect to the half-value frequency, and more important, for the slope of the curves. The disagreement of the half-value frequency could be eliminated by assuming a different value for κ_i. The lower slope requires a larger distribution in T than that calculated from the distribution in R.

To examine the influence of the size distribution on the range of relaxation times, Curve 1 of Fig. 7, for a single characteristic frequency, 3.5 Mc., is given. This is the dispersion curve of the size group with the diameter 1.5 μ. From comparison of Curves 1 and 2, it is obvious that the spectrum of the time constant caused by the distribution in R is rather small. In conclusion, the range of the time constant found experimentally cannot be explained by the measured size distribution of the mitochondria.

5. Distribution of Time Constants Resulting from Variability in the Internal Conductivity of the Mitochondria.—There are two possibilities to explain the range in T by a distribution of the internal conductance κ_i.

1. The interior is electrically isotropic, but κ_i is not identical for all mitochondria. κ_i should therefore depend on the size of the mitochondria. If the smaller mitochondria have a higher internal protein concentration, they should have a lower internal conductivity. However, a discussion similar to that advanced in the preceding paragraph discloses that a very pronounced variability of internal conductance must be assumed to explain the experimental findings (by about a factor of 4, as may be concluded from arguments forwarded in the next paragraph). Such a variability appears unlikely in view of the capability of the mitochondrial interior to adjust its conductivity proportionally with that of the exterior, as demonstrated by the data given in Table I.

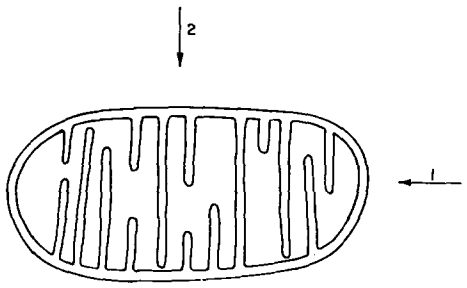

FIG. 8. Schematic figure of the parallel arrangement of the cristae of mitochondria. The preferred directions of the electric field are indicated (see text).

2. The interior is electrically anisotropic. This possibility is undoubtedly realized in case of undamaged mitochondria, due to the presence of cristae. Fig. 8 shows a schematic drawing of the arrangement of the cristae of mitochondria (Palade, 1953; Sjöstrand and Rhodin, 1953; Fawcett, 1955). The electrical conductivity of the interior in direction 1 of Fig. 8 is likely to be low, due to the insulating[3] effect of the inner mitochondrial membranes, compared to direction 2, where the electrical field is parallel to the inner membranes. If the inner membranes have a separation of approximately 500 A, then the corresponding arrangement of layers will be shortcircuited at frequencies in excess of 100 Mc. The time constant which characterizes the frequency range where the inner membranes start to be shortcircuited, is given by the product $R_M \cdot C_M$, in which C_M is the capacitance of the inner membrane and R the resistance per "compartment" separated by two successive inner membranes. Assuming C_M near 1 μf/cm.^{2} and an inner conductivity of about 10^{-3} mho/cm., which corresponds to $R_M \sim 10^{-2}$ ohm, T must be near 10^{-8} sec. This is beyond the range of the β dispersion of the outer membrane discussed so far. If the inner membrane would divide the interior of the mitochondria into completely separated compartments, the very low effective conductivity in direction 1 should shift the β dispersion caused by the outer membrane to very low frequencies, if all mitochondria were oriented

[3] The term "insulating" is used to indicate that the electrical conductance of membranes can be assumed to be very low and is not meant to imply that the inner membranes extend completely through the mitochondrion from one to the other side. The lowered internal conductance in direction 1 results from the necessity to bypass the cristae.

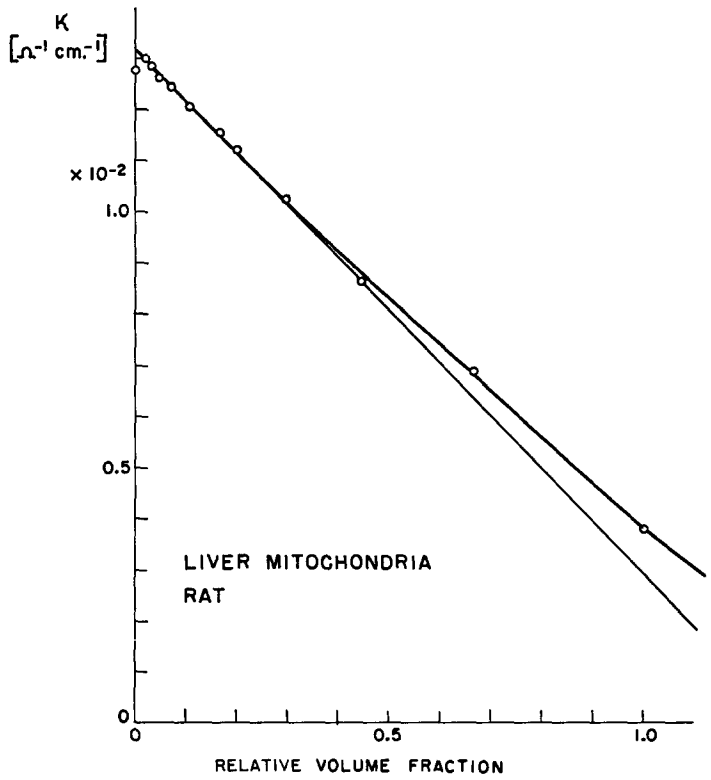

FIG. 9. Electrical conductivity of a mitochondrial suspension in 0.09 M KCl solution as function of the relative volume fraction of the mitochondrial pellet. Frequency 1 kc. Temperature 25°C.

so that direction 1 coincides with the direction of the external field. This would result, for random orientation, in relaxation time constants extending over several frequency decades.[4]

The mitochondria used for this investigation were swollen. It is known from electron microscopy (Witter, Watson, and Cottone, 1955) that only some parts of the cristae can be seen in swollen mitochondria. Nevertheless, there may well remain some electrical anisotropy of the interior.

From the random orientation of the mitochondria in the three directions possible, it is to be anticipated that about $\frac{1}{3}$ of the dielectric increment is due to a contribution of the mitochondria having the inner membranes perpendicular to the electric field. Hence the total dispersion is approximately a superposition of one-third with a lower characteristic frequency, and $\frac{2}{3}$ with a higher characteristic frequency, due to the dependence of T upon κ_i in equation (5).

Indeed, there is good agreement between experiment and the calculated curves, if we assume a 4-fold smaller conductivity for the term contributing $\frac{1}{3}$ to ϵ_0 as can be seen in Fig. 7. The part contributing $\frac{2}{3}$ to the dispersion has its characteristic frequency at 3.5 Mc.

According to the theory of dielectrics the κ dispersion is completely determined by the ϵ dispersion, due to the fact that the magnitudes of dielectric and conductivity dispersion $\Delta\epsilon = \epsilon_0 - \epsilon_\infty$ and $\Delta\kappa = \kappa_\infty - \kappa_0$ (equation 4) are related through $\epsilon_r\Delta\epsilon = T \cdot \Delta\kappa$. Indeed, the measured κ dispersion (Fig. 3, Experiment A) is in agreement with that calculated by means of the data obtained from the ϵ dispersion alone.

The above stated analysis explains also the beginning of the high frequency dispersion shown in the frequency dependence of κ in Experiment A (Fig. 3) above 50 Mc. This rise is due to the dispersion of the interior of the mitochondria. According to the theory (equation (5)) this kind of

[4] Such a spectrum of time constants is not in agreement with the presented data. This merely reflects the fact that the cristae do not completely separate the mitochondrial interior into separate compartments.

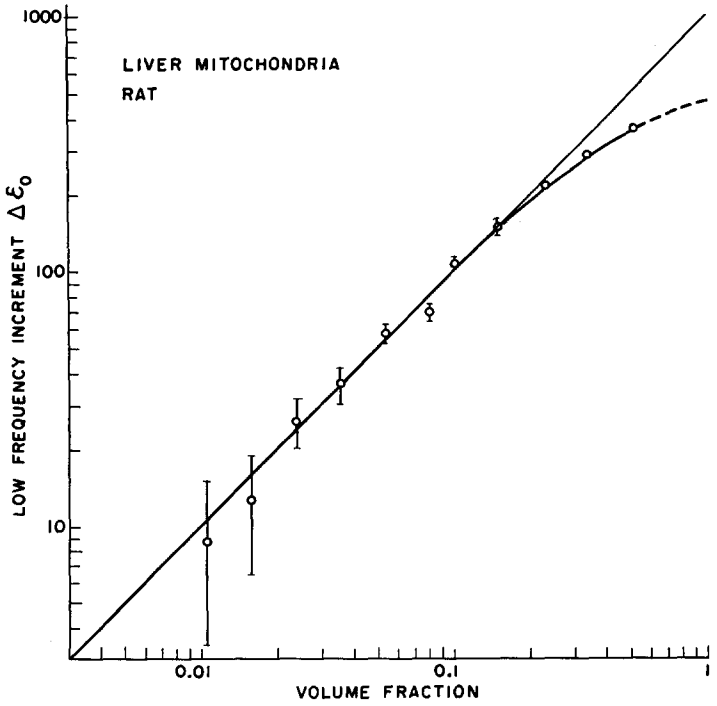

FIG. 10. Low frequency dielectric increment $\Delta\epsilon_0 = \epsilon_0 - \epsilon_\infty$ of the mitochondria suspension *versus* the actual volume fraction of the mitochondria. Frequency 0.5 Mc. Temperature 25°C. The error in the determination of the dielectric constant is indicated.

dispersion should shift to 10-fold higher frequencies; *i.e.*, above 500 Mc., if the outside medium and hence κ_i is made 10 times larger, as in Experiment B of Fig. 3. Indeed, there is no indication of any other dispersion up to 250 Mc.

6. Volume Dependence of the Low Frequency Increment.—It was mentioned earlier that equations (6) and (10) are only approximations for $p \ll 1$. In order to evaluate C_M from ϵ_0, it was necessary to introduce a factor correcting for $p \sim 0.6$. This factor was determined by measuring the low frequency conductance κ_0 and the low frequency dielectric increment ϵ_0 in a series of 11 dilutions of the mitochondrial pellet. A plot of the low frequency conductivity as function of the relative volume fraction is shown in Fig. 9. A value of 1 corresponds to the undiluted pellet. Using equation (8), an absolute volume fraction of 0.53 for the sediment was calculated for this case.

Using the value for the absolute volume fraction of mitochondria obtained above, the plot of the low frequency dielectric increment as function of the volume fraction was obtained (Fig. 10). It can be seen that the linearity predicted by equations (6) and (10) holds for $p < 0.2$. The correction factors used to evaluate C_M from ϵ_0 were derived from this plot.

INTERPRETATION OF MEMBRANE CAPACITANCE

Earlier studies on the electrical properties of cells have yielded values between approximately 0.5 and 1.5 μf./cm.^{2} for membrane capacity. It is remarkable that similar values for membrane capacity have been obtained from such widely different cell types as plants, erythrocytes, eggs, bacteria, and nerve and muscle fibres. A mitochondrial membrane capacity of 0.5 to 0.6 μf./cm.^{2} found in this investigation falls within the range of values obtained for other membranes. It is suggested here that the similarity in membrane capacity between isolated mitochondria and intact cells is indicative of a structural similarity. It may well be that a universal molecular structure of which membranes are composed, is responsible for this fact (Schwan, 1957).

The presence of lipids in cell membranes has been known for some time. For example, the presence of lipid is an integral part of erythrocyte ghosts. The treatment of this membrane structure with lipid-reacting components such as saponin and digitonin (Fricke and Curtis, 1935) destroys the electrical capacity of the membrane, suggesting

again that lipid is an essential component of the membrane. The latter had been proposed by Overton at the end of the last century because of the close correlation between lipid solubility and permeability of substances (*cf.* also Davson and Danielli, 1952). In this regard the determination of the capacity of artificial lipoprotein films; *e.g.*, bimolecular egg albumin and lecithin (Dean, Curtis, and Cole, 1940) requires mention. The authors found a value for this "membrane capacity" of 1.1 to 1.2 μf./cm.^{2}

The relationship of membrane capacitance C_M and thickness d is expressed by $C_M = \epsilon_r \epsilon_M / d$ in which ϵ_M is the dielectric constant of the membrane. If the membrane or that part of it which manifests itself in C_M would be composed only out of lipids, its dielectric constant ought to be rather small, between 3 and 6. The corresponding thickness would be then about 30 A (Fricke, 1925) to 60 A. The latter value is in reasonably close agreement with the thickness of a double leaflet of lipids within the myelin structure (Geren and Schmitt, 1955). But since no dielectric data for the membrane lipids are available, the agreement may be coincidence.

It seems to be reasonable in consideration of the results of electron microscopical and x-ray diffraction studies with myelin lamellae (Geren and Schmitt, 1955; Finean and Robertson, 1958; Robertson, 1958), to take cognizance of the protein content of the membrane, too. We know from recent measurements on hydrated protein systems that the effective dielectric constant of a hydrated protein is about 5- to 10-fold higher than that quoted above for lipids, throughout the frequency range of interest here (Schwan, 1957).

The contribution of the protein part of the membrane to C_M depends on its dielectric constant and on its conductivity. Since no data are available—except the mentioned ϵ values for hydrated protein—it is not possible to give a reliable figure for the over-all thickness of the membrane from C_M alone. An upper limit would be obtained if the membrane would be composed of protein matter only. Its thickness corresponding to a C_M of 1 μf./cm.^{2} would be about 300 A. Further knowledge is to be gained from investigations of membranes of known thickness and from dielectric data of protein solutions. In view of the rather limited knowledge of the dielectric properties of the membrane substances, and considering the range from 0.5 to 2 μf./cm.^{2} of C_M values previously reported for cell membranes, it is not possible to state that the mitochondrial membrane is a single or a double membrane. If double, the membrane of the mitochondrion would be composed of two double leaflets of lipid molecules. Electron micrographs support the latter structure (Palade, 1953; Robertson, 1958; Fernández-Morán, 1959). In any case, it can be said on the basis of the above presented data that the electrical properties of the mitochondrial and cellular membrane are quite similar.

We wish to express our appreciation to Dr. Britton Chance for his interest in this work and for the use of the facilities of the E. R. Johnson Foundation for Medical Physics.

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