Electrical Properties of Mitochondrial Membranes*

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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

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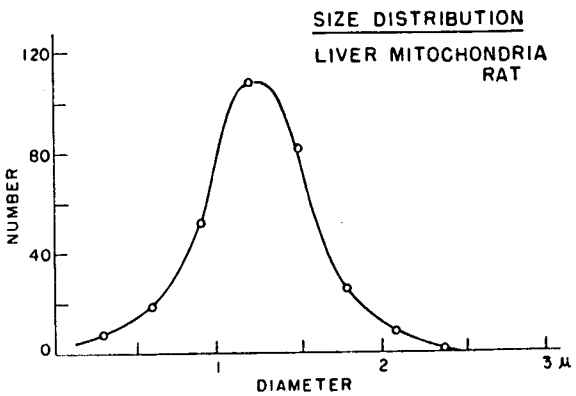

FIG. 1. Size distribution of rat liver mitochondria after swelling in 0.001 M KCl solution and equilibration in 0.012 M KCl solution (Experiment A) or 0.13 M KCl solution (Experiment B).

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-

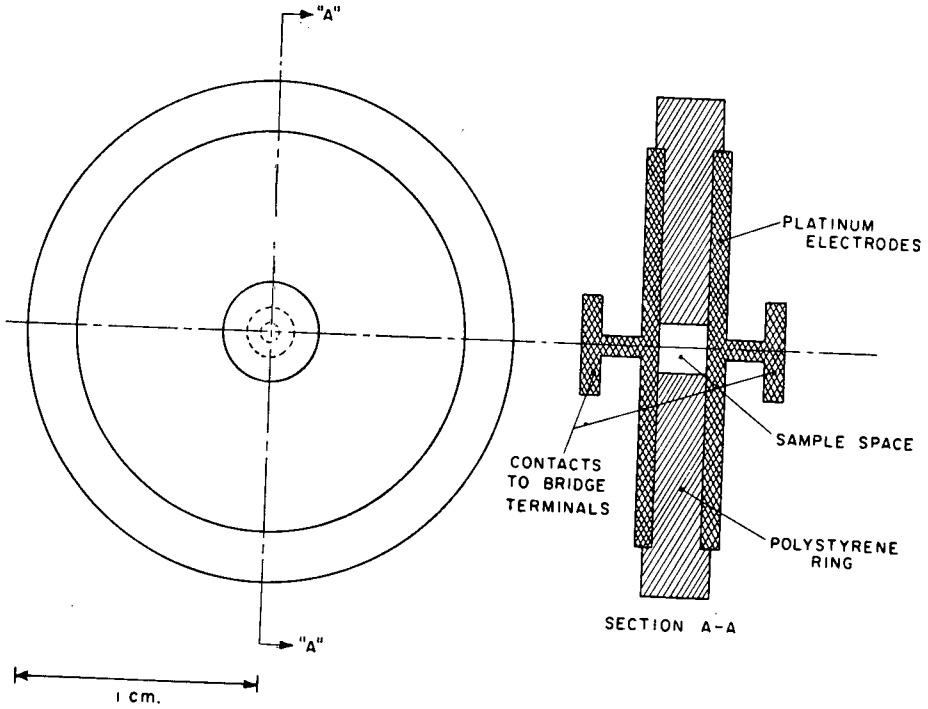

FIG. 2. Cell for dielectric measurement. For description see text.

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.

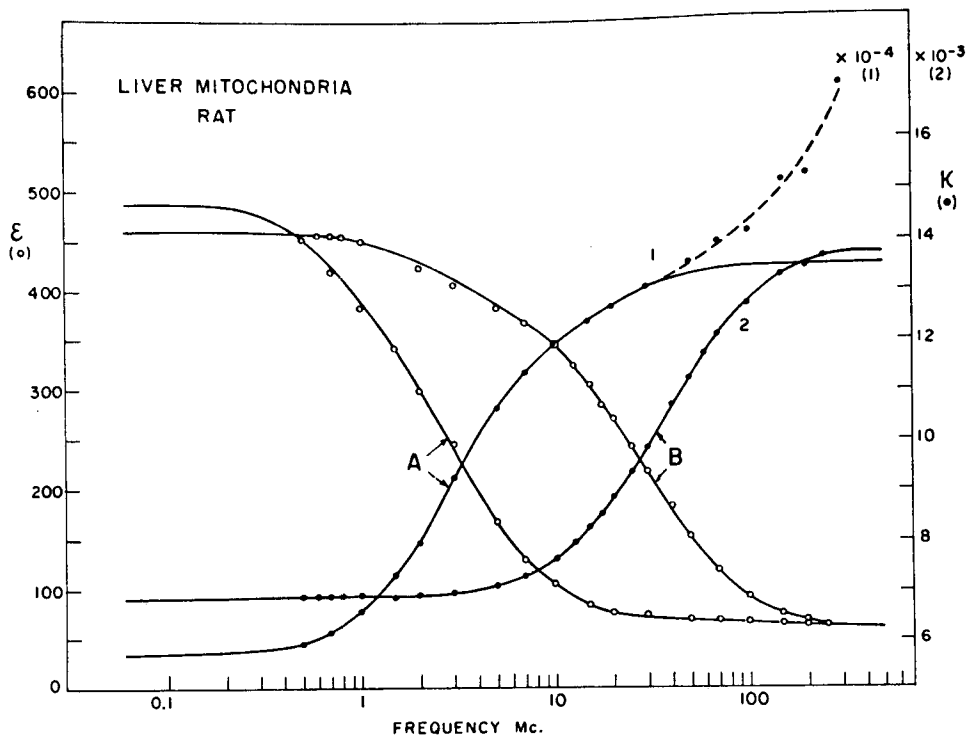

FIG. 3. β-dispersion of swollen rat liver mitochondria.

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.

Analysis of Data

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

[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).

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):

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

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

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

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

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

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)).