The Relationship of Internal Conductance and Membrane Capacity to Mitochondrial Volume*

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ABSTRACT

A study was made of the effect of mitochondrial size on the electrical properties of the membrane and the internal conductivity of mitochondria. The dielectric constant and electrical conductivity of suspensions of guinea pig heart mitochondria were examined in the frequency range 5×10^5 to 2.5×10^8 C.P.S.

Membrane capacity was calculated to be 1.1 to 1.3 μf./cm.^{2} and was virtually the same in mitochondria whose surface area was made to vary by a factor of 4 by osmotic means. This finding suggested that some mechanism must exist for the transfer of mitochondrial material into membrane structure during fluctuations in mitochondrial size.

The electrical capacity of the membrane was unaffected by a 33-fold change in potassium chloride concentration.

The internal conductance of swollen mitochondria was 2 to 3 times lower than that of the external medium. In shrunken mitochondria the internal conductance was virtually independent of the conductivity of the external medium.

These results were discussed in relation to current concepts of mitochondrial structure.

INTRODUCTION

In a preceding paper, it has been shown that the membrane of rat liver mitochondria has an electrical capacity of 0.5 to 0.6 μf./cm.^{2} (Pauly, Packer, and Schwan, 1960). Since the membrane capacity of this intracellular particle is approximately the same as that known for the cell membrane, it was suggested that a universal molecular structure may exist in biological membranes.

It is well known that mitochondria change their size and shape *in vivo* (Frédéric, 1954; Chévremont and Frédéric, 1956) in relation to the metabolic activity of the cell. Such structural changes may play a significant role in the semipermeable nature of the membrane. Since it is possible to determine the electrical capacity of mitochondrial membrane and the conductivity of the interior of mitochondria, we decided to examine the influence of mitochondrial size on membrane structure and some of the permeability aspects relating to the mitochondrial interior. The electrical capacitance reflects the presence of the bulk of molecular components which establish the membrane structure, while the permeable function of the membrane is probably determined by "holes" which occupy only an extremely small fraction of the total membrane surface. Thus part of this study is concerned with an investigation into the effects of volume changes of mitochondria with regard to the bulk of the membrane matter. The conductivity investigation is concerned with the size and internal composition of mitochondria in relation to their environment and undertaken to gain additional information about the factors which determine the amount of freely movable ions inside cells and subcellular particles.

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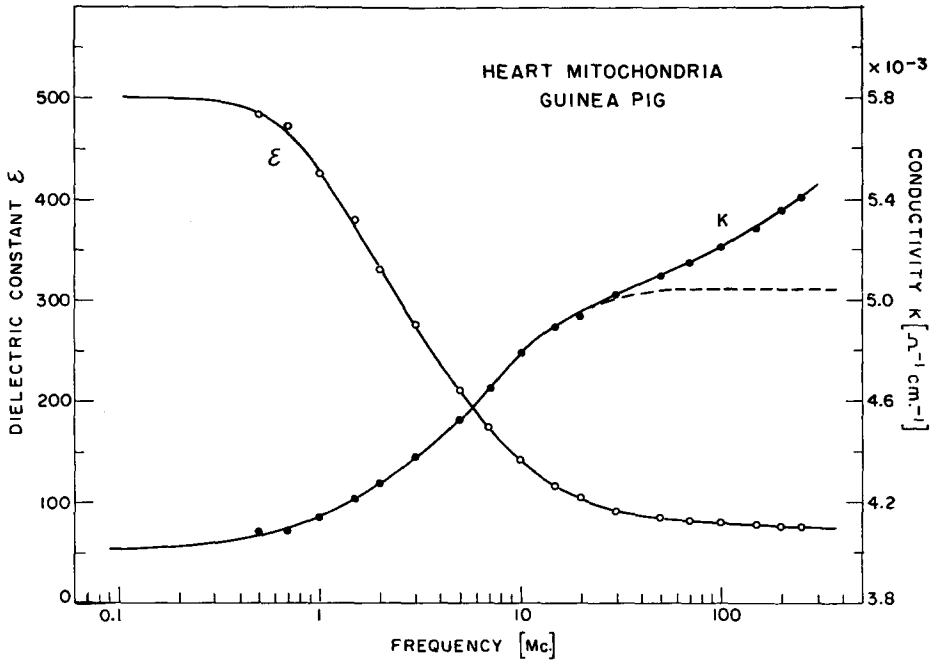

FIG. 1. Anomalous dispersion of a suspension of swollen guinea pig heart mitochondria. 0.054 M KCl; temperature + 4°C.

Methods

Guinea pig heart mitochondria were isolated in sucrose (0.32 M)-versene (0.001 M) medium by the procedure recommended by Cleland and Slater (1953) for rat heart. The mitochondria were washed four times in the isolation medium. The final mitochondrial residue was diluted in a small aliquot of isolation medium and glutamate (10 mM) was added. It was necessary for these experiments to keep the mitochondrial volume constant. Since the rate of swelling of mitochondria which accompanies aging is decreased by anaerobic conditions (Hunter, Davis, and Carlat, 1956; Price, Fonnesu, and Davis, 1956; Lehninger and Ray, 1957; Packer, 1958), glutamate was added as an oxidizable substrate to remove the dissolved oxygen of the medium as the washed mitochondria are almost devoid of endogenous respiration. An additional precaution was to carry out the experiments at a constant temperature of 4°C.

To examine the effect of increasing the mitochondrial volume on the electrical properties of the membrane, the following procedure was adopted. A small aliquot of the above mentioned concentrated mitochondrial suspension was diluted in a large volume of KCl (0.01 M) and allowed to stand overnight at 4°C. Following this the suspension was washed twice by centrifuging at 10,000 × *g* for 10 minutes in KCl (0.01 M). The mitochondrial pellet was finally suspended in a small aliquot of KCl (0.01 M).

The conductivity of the external medium was varied in both the "shrunken" and swollen mitochondria by the addition of small amounts of a 3 M KCl solution. Experiments were carried out 4 hours later to permit equilibration of internal and external electrolyte. Size distribution curves were obtained for the "swollen" and "shrunken" mitochondria with the phase contrast microscope (*cf.* Pauly, Packer, and Schwan, 1960).

The admittance of the sample was measured with the "RX-Meter type 250-A" of the Boonton Radio Corporation, Boonton, New Jersey. The experimental procedure is described in a preceding paper (Pauly, Packer, and Schwan, 1960).

RESULTS

Capacitance of Membrane:

The frequency dependence of ϵ and κ of swollen heart mitochondria in 0.003 M KCl is shown in Fig. 1. The results are similar to those reported previously for liver mitochondria, including the beginning of the high frequency dispersion recognizable at the high frequency end of the κ dispersion, due to internal structure.

A dielectric plot (Cole and Cole, 1941) of the data is given in Fig. 2. The measured points yield a semicircle with a slightly depressed center. This indicates that the dispersion deviates somewhat from the one described by a Debye-term with a

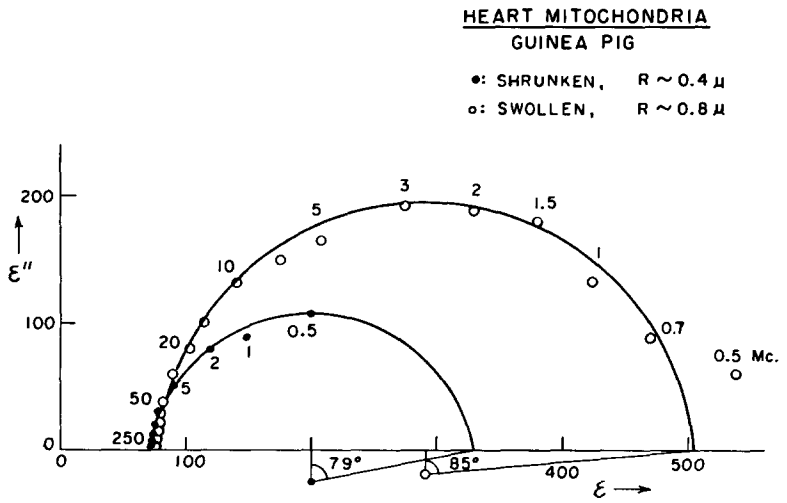

FIG. 2. Plot of $\Delta\kappa/\omega\epsilon_r$ versus ϵ for swollen and shrunken heart mitochondria in the complex dielectric plane. Frequencies are indicated in Mc. (See Table I, Sample 2).

(○) Swollen mitochondria; 0.054 M KCl,

(●) Shrunken mitochondria; 0.018 M KCl,

0.001 M versene, 0.01 M glutamate, anaerobic condition, temperature + 4°C. (See Table II, Sample 2).

single time constant. From the dielectric plane the values for low frequency DK (dielectric constant) ϵ_0 and the high frequency DK ϵ_∞ were obtained by extrapolation to the abscissa.

It can be seen from Fig. 2 that the extrapolation in the dielectric plane gives more reliable values for ϵ_0 and ϵ_∞ in the case of swollen mitochondria, than in the case of "shrunken" mitochondria. In the latter, the extrapolation must extend over a larger frequency range, as is obvious from the plot for shrunken mitochondria and results in a greater uncertainty as to exact value of ϵ_0.

The influence of the range of relaxation times on the dispersion curves is shown in Fig. 3. In Curve *1*, ϵ_1, and κ_1 refer to a curve characterized by a single time constant. Curve *2* is that for swollen mitochondria (experiment from Fig. 1). Curve *3* refers to shrunken mitochondria. In order to facilitate comparison and display the influence of the presence of a spectrum of relaxation times the κ and ϵ curves, as well as the frequency, were normalized. The "normalized" frequency is $f/\sqrt{f_{0\kappa} \cdot f_{0\epsilon}}$ in which $f_{0\epsilon}$ is the half-value frequency for ϵ (this means the frequency with which $\frac{\epsilon - \epsilon_\infty}{\epsilon_0 - \epsilon_\infty} = \frac{1}{2}$), and $f_{0\kappa}$ the corresponding frequency for κ. In this presentation the corresponding ϵ and κ curves intersect near unity. The broader the spectrum of time constants, the lower the intersection. It can be seen that shrunken mitochondria show a low intersection, indicative of a broader spectrum of time constants than is present in the case of swollen mitochondria.

The mitochondrial membrane capacity C_M was evaluated by means of equations discussed in the preceding paper (Pauly, Packer, and Schwan, 1960).

$$C_M = \frac{4\epsilon_r}{9} \frac{\epsilon_0 - \epsilon_a}{\sum_i p_i R_i} \tag{1}$$

$$C_M = \frac{1}{\pi f_0 \bar{R}} \frac{\kappa_i \kappa_a}{\kappa_i + 2\kappa_a} \tag{2}$$

in which C_M = membrane capacity per cm.^{2} (farad/cm.^{2});

ϵ_0 = low frequency dielectric constant;

ϵ_a = dielectric constant of the suspending medium;

ϵ_r = dielectric constant of the vacuum ($\frac{1}{4}\pi \cdot 9 \cdot 10^{11}$);

κ_i = specific conductivity of the mitochondrial interior (ohm^{-1} cm.$^{-1}$);

κ_a = specific conductivity of the suspending medium (ohm^{-1} cm.$^{-1}$);

$\bar{R}$ = radius of the mitochondria (cm.);

p_i = volume fraction of the mitochondria with the radius R_i;

$\bar{R}$ = "effective" radius of the mitochondria; in this case the radius of the volume fraction p_i of the size distribution with the largest contribution to ϵ_0;

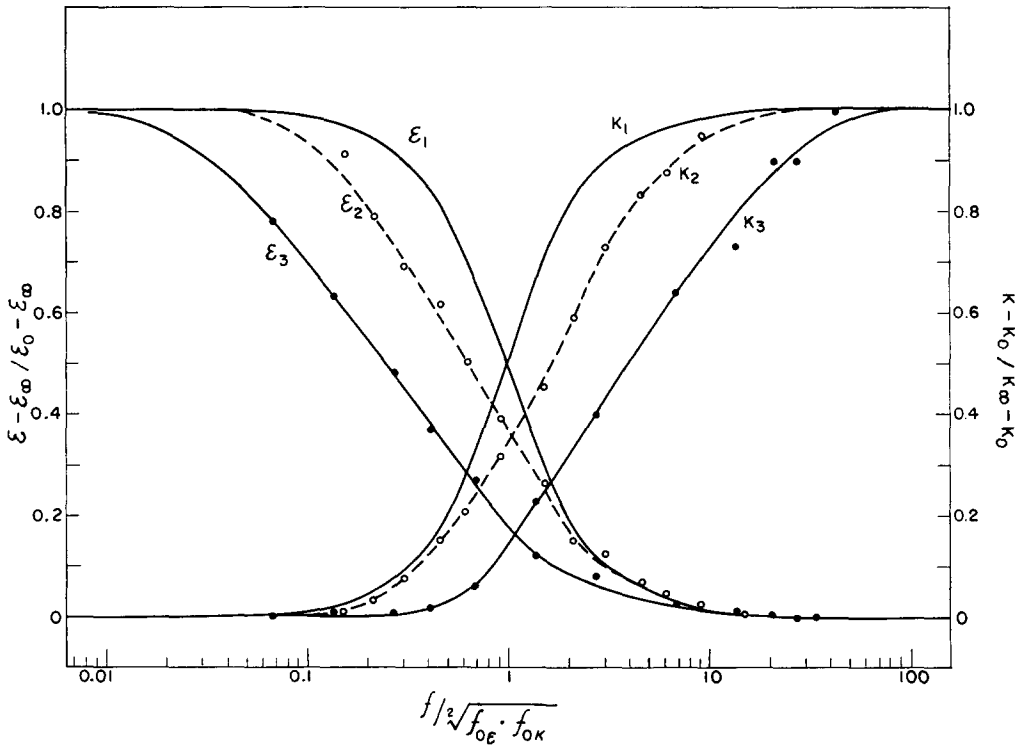

FIG. 3. Normalized dispersion curves for a single relaxation time constant (ϵ_1, κ_1), for swollen mitochondria (ϵ_2, κ_2), and for shrunken mitochondria (ϵ_3, κ_3). For explanation see text.

f_0 = "characteristic" frequency. This is, in the case of a single relaxation time, the frequency in which the normalized functions $\frac{\epsilon - \epsilon_\infty}{\epsilon_0 - \epsilon_\infty} = \frac{1}{2}$ and $\frac{\kappa - \kappa_0}{\kappa_\infty - \kappa_0} = \frac{1}{2}$.

In the case of a distribution of relaxation times no general theory is available. A reasonable compromise may be the geometrical means of $f_{0\epsilon}$ and $f_{0\kappa}$ or $f_0 = (f_{0\epsilon} \cdot f_{0\kappa})^{1/2}$, in which $f_{0\epsilon}$ is the half-value frequency of ϵ; and $f_{0\kappa}$ that of κ.

In the case of a broad spectrum of relaxation times, the value of the membrane capacity evaluated from equation (2) is considered somewhat less reliable than the value calculated from the extrapolated low frequency dielectric constant ϵ.

Equations (1) and (2) are approximations. The following assumptions were made:

1. The thickness of the membrane is very small compared to the radius R;

2. The conductance of the membrane is small compared to that of inner and outer phase;

3. The volume fraction $p \ll 1$;

4. The interior is isotropic with respect to electrical properties.

Assumptions 1 and 2 appear justified unless the membrane conductivity is much larger than 0.1 mho/cm.^{2}, *i.e.* much in excess of values known for other membranes (Cole and Curtis, 1950).[1] A value of 0.1 mho/cm.^{2} corresponds to a specific resistance of 10 million ohm cm. for a membrane 100 A thickness. This specific resistance is large compared to the specific resistances of inner and outer phase, *i.e.* values between 100 and 1000 ohm cm.

The third assumption is fulfilled, since the volume fractions used were $\sim$0.15.

The fourth assumption is obviously not fulfilled in the case of "shrunken" mitochondria, due to the presence of the insulating cristae mitochondriales. In swollen mitochondria these cristae are partially broken down. Anisotropy is much reduced but still noticeable. This was shown with liver mitochondria. The anisotropy of the interior is believed to contribute greatly to the spectrum of relaxation times described above (Pauly, Packer, and Schwan, 1960).

[1] This may readily be seen from a discussion of formulae given previously (Schwan, 1957, 162, equation (19)).

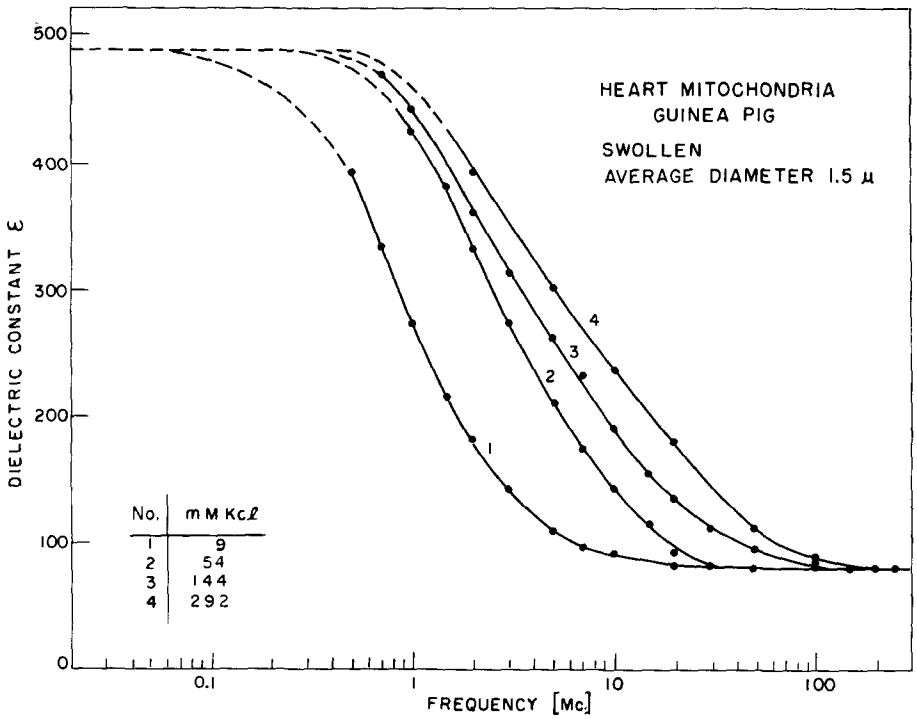

FIG. 4. Dielectric constant of 4 suspensions of swollen mitochondria as function of frequency. The KCl concentrations of the 4 suspensions are indicated in the figure. (For further data see Tables I and III).

The volume concentration was determined electrically using the formula

$$\frac{\kappa_0}{\kappa_a} = \frac{1 - p}{1 + \frac{p}{2}} \tag{3}$$

in which κ_0 is the low frequency conductivity (mho/cm.) and p and κ_a as defined above.

The conductivity of the mitochondrial interior was determined by:

$$\frac{\kappa_\infty}{\kappa_a} = \frac{1 + 2p\,\frac{\kappa_i - \kappa_a}{\kappa_i + 2\kappa_a}}{1 - p\,\frac{\kappa_i - \kappa_a}{\kappa_i + 2\kappa_a}} \tag{4}$$

in which κ_∞ is the high frequency conductivity (mho/cm.) and the other symbols have the definitions given earlier. Equations (3) and (4) have been given by Maxwell (1892).

In Figs. 4 and 5 the dielectric constant of a suspension of shrunken and swollen mitochondria at 4 different KCl concentrations is plotted as a function of frequency. As predicted by equation (2), the dispersion shifts to higher frequencies with increasing ionic concentration. The extrapolated dielectric constant ϵ_0 at low frequency was found to be independent of ionic strength, as predicted by equation (1). Hence the general dielectric behavior of a suspension is rather well described by the theory of dielectric behavior of particles surrounded by a membrane. Since the low frequency dielectric increment is proportional to the radius R of the mitochondria, ϵ_0 is smaller in the case of shrunken mitochondria. Another feature of shrunken mitochondria is the wider spectrum of relaxation times already mentioned in connection with Fig. 3.

The membrane capacity was evaluated by means of equations (1), (2), (3), and (4). The terms $\Sigma p_i R_i$ in equation (1) were obtained from size distribution curves for shrunken and swollen mitochondria and from the total volume concentrations determined from equation (3) (*cf.* Pauly, Packer, and Schwan, 1960).

The result of the analysis for swollen mitochondria is summarized in Table I. The average value for the membrane capacity calculated from ϵ_0 by equation (1) is 1.3 μf./cm^2. The value does not vary by more than 10 per cent. As expected,

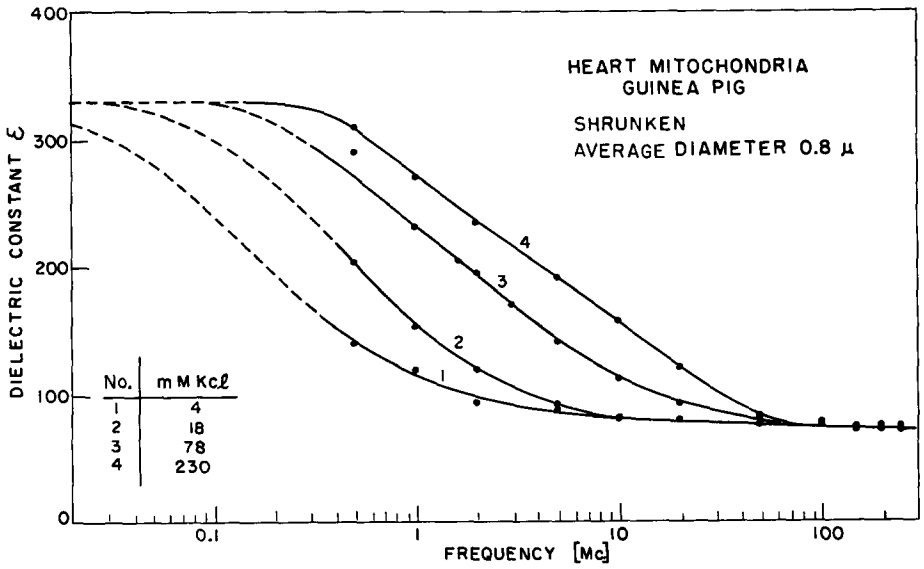

FIG. 5. Dielectric constant of 4 suspensions of shrunken mitochondria as function of frequency. The KCl con centrations of the 4 suspensions are indicated in the figure. (For further data see Tables II and III).

TABLE I

Membrane Capacity Guinea Pig Heart Mitochondria Swollen, Average 1.5 μ

Summary of data and membrane capacity values of swollen guinea pig heart mitochondria. For explanation see text.

Sample No.	KCl	κ_α	κ_0	ϵ_0	ϵ_∞	$f_0 = (f_{0\epsilon} \cdot f_{0\kappa})^{1/2}$	C_M from ϵ_0	C_M from f_0
	mM	*m mho/cm.*	*m mho/cm.*			*Mc.*	*μf./cm.²*	*μf./cm.²*
1	8.6	0.85	0.68	500	78	1.5	1.4	0.5
2	54.5	4.9	4.0	500	78	4.1	1.4	0.7
3	144	12.6	9.7	450	78	8.0	1.2	0.8
4	292	24.6	20.7	450	70	9.6	1.3	1.2
Average							1.3	0.8

~15 Volume-per cent in KCl-solution, temperature + 4°C.

the values for C_M, evaluated from the average characteristic frequency f_0 by means of equation (2) are less reliable. There is a systematic shift with the KCl concentration of the suspending fluid, the cause of which is unknown. Nevertheless, the C_M values, calculated from f_0 compare roughly with those obtained from ϵ_0. The deviations are believed to be related to the presence of time constant spectra as discussed previously. A systematic shift of C_M with tonicity evaluated from f_0 was found for shrunken mitochondria also (Table II). Even here the values are of the same order of magnitude as the values calculated from the extrapolated value of ϵ_0 (*cf.* Fig. 2). The good agreement between the average of C_M (ϵ_0) with that of C_M (f_0) of 1.1 μf./cm.² must be considered a coincidence. The error inherent in the final values could be easily as large as 30 per cent, so that C_M for shrunken mitochondria lies between 0.8 and 1.4 μf./cm². Hence it can be stated that the membrane capacity of guinea pig heart mitochondria is about 1.1 to 1.3 μf./cm.², the value being somewhat higher than that given previously for liver mitochondria.

Internal Conductance of the Mitochondria:

The electrical conductivity of an electrolyte is determined by its ion concentration and mobility. Therefore, electrical conductivity should provide information about the state of the ions within the

TABLE II

Membrane Capacity Guinea Pig Heart Mitochondria Shrunken, Average Diameter 0.8 μ

Summary of data and membrane capacity values of shrunken guinea pig heart mitochondria. For explanation see text.

Sample No.	κ_a	κ_i	$f_0 = (f_{0\epsilon} \cdot f_{0\kappa})^{\frac{1}{2}}$	C_M
	m mho/cm.	*m mho/cm.*	*Mc.*	*μf./cm.²*
1	0.43	0.6	0.7	1.8
2	1.79	1.1	2.0	1.5
3	7.04	1.5	6.8	0.7
4	19.7	1.6	16.2	0.4
Average				1.1 μf./cm.²
Membrane capacity from ϵ_0				1.1 μf./cm.²

~ 15 volume-per cent in 0.32 M sucrose, 0.001 M versene, 0.01 M glutamate + KCl, anaerobic, temperature + 4°C.

mitochondria. Since a high degree of accuracy was desired, the internal conductance was measured in a packed sediment of mitochondria. The extramitochondrial space was evaluated by means of equation (3) from the low frequency conductivity and the conductivity of the suspending medium. The extramitochondrial space was found to be 40 per cent. With this value and the high frequency conductance κ_∞ the internal conductivity κ_i was evaluated by means of equation (4). κ_∞ was obtained from the conductivity at 100 Mc., using a small correction, extrapolating the β dispersion to infinite frequencies. The data are shown in Table III. Internal conductivity as function of the conductivity of the suspending medium is plotted in Fig. 6. It can be seen that the swollen and shrunken mitochondria behave differently. The conductivity of the swollen mitochondria is 2 to 3 times lower than that of the external medium. The same relation was found in blood cells (Fricke and Morse, 1926; Fricke and Curtis, 1934; Pauly, 1959), liver cells (Schwan, 1958), and nerve fibres (Hodgkin and Keynes, 1953). This lower conductivity probably is due in good part to the relatively high content of proteins and lipids. The presence of protein molecules will lower the conductance by their volume requirement and by binding of ions. The latter mechanism is indicated by the measurements of Bartley and Davies (1954). The former mechanism is indicated by the investigations of Tedeschi and Harris, (1955), who reported an osmotically inactive space of 40 to 50 per cent in rat liver mitochondria.

For swollen mitochondria, κ_i/κ_a changes by only a factor of 2, while the external conductivity changes by a factor of 33. Hence the mitochondrial interior and the external medium appear to be well equilibrated. This is clearly shown in Fig. 6.

The different behavior of shrunken mitochondria may be expected from the fact that the average volume is 6 to 7 times smaller than in swollen mitochondria. Hence, the concentration of proteins and lipids is nearly 6 to 7 times higher. Table III shows that in the most dilute sample the internal conductivity is 1.4 times higher than that of the external medium. This means a mitochondrion in this state is able to maintain a certain ionic composition in the presence of an electrolytic concentration gradient across the mitochondrial membrane. The concentration gradient is actually much larger than reflected by the conductivity ratio 1.4, in part due to the fact that the mobility and the space available for the ions is restricted and the ionic concentration within the mitochondria is correspondingly greater. The tendency to keep the internal ion

TABLE III

Internal Conductance Guinea Pig Heart Mitochondria

Summary of internal conductivity data of swollen and shrunken heart mitochondria. For explanation see text.

Swollen, average diameter 1.5 μ						Shrunken, average diameter 0.8 μ					
Sample No.	KCl	κ_a	κ_∞	κ_i	$\frac{\kappa_i}{\kappa_a}$	Sample No.	KCl	κ_a	κ_∞	κ_i	$\frac{\kappa_i}{\kappa_a}$
	mM	*m mho/cm.*	*m mho/cm.*	*m mho/cm.*			*mM*	*m mho/cm.*	*m mho/cm.*	*m mho/cm.*	
1	8.6	0.85	0.69	0.55	*0.64*	1	4	0.43	0.5	0.6	*1.4*
2	54	4.9	3.0	2.0	*0.41*	2	18	1.79	1.3	1.1	*0.6*
3	144	12.6	6.8	3.8	*0.30*	3	78	7.04	3.3	1.5	*0.2*
4	292	24.6	13.2	7.6	*0.32*	4	230	19.7	7.3	1.6	*0.08*

Packed in KCl-solution, volume fraction $p \sim 0.6$, temperature ~ +4°C.

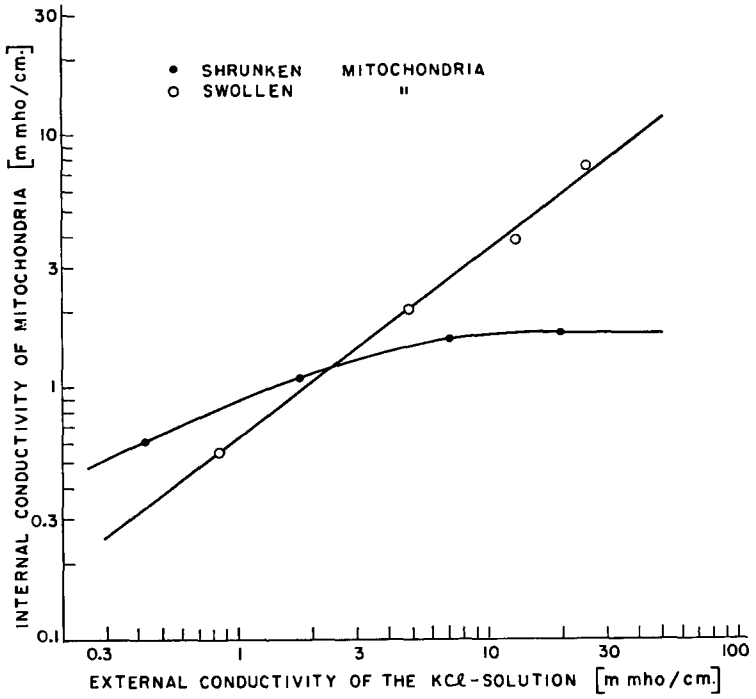

FIG. 6. Internal electrical conductivity of swollen and shrunken mitochondria as function of the electrical conductivity of the suspending medium. (For complete data see Table III).

milieu constant is suggested also by the internal conductance values at higher ionic concentrations of the suspending medium (Samples 2 to 4). In a solution of 0.23 M KCl and 0.32 M sucrose, the internal conductance is as much as 12 times lower than the external conductance. The absolute values of the internal conductance κ_i as plotted against κ_a in Fig. 6, show clearly that at higher ionic concentrations in the suspending medium the mitochondrial interior is nearly independent of κ_a .

In summary: Swollen mitochondria approach a system of complete equilibration of inner and outer phase, whereas shrunken mitochondria try to maintain their internal ionic composition constant. These two examples establish limiting cases of a more general equilibration of the Donnan-type. In the swollen mitochondria the volume fraction of the macromolecules is quite small. With shrunken mitochondria the influence of the comparatively large volume fraction of the macromolecules with their fixed charges and counter ions is so predominant that the small remaining osmotically active space is of minor importance to the behavior in an electrolytic external medium of varied tonicity.

DISCUSSION

The membrane capacity of guinea pig heart mitochondria was found to be 1.1 μf./cm.^{2} for shrunken mitochondria, and 1.3 μf./cm.^{2} for swollen mitochondria. Since the error could readily be as large as 30 per cent in the former case and 10 per cent in the latter, both values are the same within the limit of error.

Electron microscopical observations indicate that the mitochondrial membrane is composed of two distinct single membranes (Palade, 1953, 1956; Robertson, 1958; Fernández-Moran, 1959).

They suggest further that the surface of the external membrane becomes enlarged during swelling, since there is no evidence for folds in the external membrane. At the same time, the mitochondrial volume increases considerably (Palade, 1958; Witter, Watson, and Cottone, 1955; Lever and Chappell, 1958; Watson and Siekevitz, 1956).

There are indications that the internal membrane, of which the cristae mitochondriales are a part, may break up. Parts of the internal material, including the rest of the cristae material are attached to one side of the external membrane. This effect may be seen in the phase contrast microscope

in the form of a darker crescent in the vesicle-like swollen mitochondria (Frédéric, 1954; Harman, 1956).

In shrunken mitochondria, both membrane capacities are arranged in series. Hence the disappearance of the internal membrane in the swollen state results in an increase in membrane capacity by a factor of 2, if the inner and outer membranes have the same capacity.

The surface area of the external membrane of the swollen mitochondria was found to be 4 times larger than in the shrunken state, reflecting a change in diameter by a factor of 2. If the membrane were stretched so that the same amount of membrane material had to cover a surface area 4 times larger, the thickness of the membrane should be expected to decrease by about a factor of 4. This should increase the membrane capacity by about the same factor.

Thus both effects cited above, when taken together, should result in a membrane capacity 8 times larger for swollen mitochondria. The measurement—even considering errors of up to 30 per cent—clearly rules this out. Therefore, the experimental results make a stretching of the membrane extremely unlikely. This result is supported by the general knowledge of the physics of surface films. We conclude: A mechanism for the transfer of mitochondrial material into membrane during the swelling process seems to exist.

The second process—the rupture of the internal membrane during swelling—cannot definitely be ruled out. The error of about 30 per cent in the shrunken mitochondria and the approximate error of 10 per cent in the swollen mitochondria permit a small possibility for a change in the over-all capacity of the membrane during swelling. The suspension of swollen mitochondria could be a mixture of mitochondria with the original double membrane and a smaller fraction of mitochondria with internal membranes ruptured from swelling.

In an electron microscope study of rat liver mitochondria, Schulz, Löw, Ernster, and Sjöstrand, (1956) found in thyroxin-treated animals that the mitochondria had swollen, with a 3- to 4-fold increase in surface of the outer membrane. The average number of the inner membranes was reduced and the length of an inner membrane became shorter. In spite of the extensive change in structure, the 150 A thickness of the outer membrane was practically unchanged. This result indicates also that the new outer membrane was formed from material originating from the inner membranes.

The dynamic aspects of the mitochondria in living cells were emphasized by Frédéric, (1954), and Chévremont and Frédéric (1955). They showed that the size, form, and number of the mitochondria in living fibroblast cultures undergo steady changes. Frequently fusion of several mitochondria into one, and refragmentation into smaller units were observed. The dynamic nature of the mitochondria reflects a dynamic nature of the mitochondrial membrane. In this respect, the mitochondrial membrane seems to behave like cytoplasmic membranes, as could be shown by Fawcett and Ito (1958), with phase contrast and electron microscopy. These authors call attention to the pronounced changes in the endoplasmic reticulum and especially to the cisternae in the living testicular cell. They found that new cisternae "have been formed by coalescence and reorganization of the tubular elements." Of special interest in this respect are the "electron micrographs of myelin figures of phospholipide simulating intracellular membranes" by Revel, Ito, and Fawcett (1958). The artificial membranes obtained by hydration of mixtures of phospholipids were strikingly similar to the double-edged cellular membranes. They consist of two dense lines, 25 to 30 A wide, separated by a less dense space of the same width.

The membrane capacity is a function of the dielectric constant ϵ_M and the thickness d of the membrane. In order to obtain the thickness, an assumption about ϵ_M has to be made (Pauly, Packer, and Schwan, 1960). Electron microscopic studies, permeability, x-ray diffraction, optical polarization, and the work on surface films suggest that the membrane model by Danielli (Davson and Danielli, 1952) is the most reasonable, at least in the present state of our knowledge. This model would explain the quick appearance of new membranes, since the double leaflet is a film, and new material can easily be added, as in the well known surface films. The protein layer on both sides of the film seems to be adsorbed, like the adsorption of proteins at the air-water surface.

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