2,4 Dinitrophenol and the Permeability of Mitochondria

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Bielawski, Thomas and Lehninger (1966) report a decrease in the electrical resistance of phospholipid bilayers brought about by the addition of 2,4 dinitrophenol (DNP). The DNP-induced effect reduces the resistance to as little as 0.4% of the original value. The concentration for a half maximal effect was found to be 0.5 mM. The difference in the concentration required for this effect and that required to uncouple oxidative-phosphorylation was attributed to the uptake of the DNP by the lipid solution surrounding the aperture and the difference in composition between the mitochondrial and the synthetic membrane.

This experiment was considered to be a test of one of the elements of the Mitchell chemi-osmotic hypothesis of oxidative-phosphorylation coupling (e. g. Mitchell, 1966). However, it was recognized that the hypothesis predicts an increase in proton permeability only.

Experiments carried out in this laboratory in the course of a systematic study of variations in mitochondrial permeability have some bearing on this question. The photometric technique of Tedeschi and Harris (1958) was used. The results of our experiments preclude a large change in the permeability to malonamide and sodium acetate induced by relatively high concentrations of DNP (0.1 mM). Substantive uncoupling takes place at lower concentrations, for example, Parker (1965) reports 50% uncoupling by .03 mM DNP.

In the present study, the changes in volume with time differ depending on whether or not the particles have been exposed to DNP (the DNP

preparations swell less). However, these effects can be attributed to unrelated changes in volume, such as a leakage of internal solute.

a. Rationale of the method

Mitochondria swell in response to the penetration of solute. Since they behave as an osmotic system (e. g. Tedeschi and Harris, 1955), the entrance of penetrant must be followed by that of water. From the volume changes, it is rather simple to calculate the amount of solute transferred (Tedeschi and Harris, 1958). From the kinetics of the volume change, it is also possible to calculate permeability constants directly (see Jacobs (1952), Tedeschi, 1959). This approach assumes (a) osmotic behavior, (b) osmotic equilibrium at all times (i.e. a high permeability to water; see Tedeschi and Harris (1958), Bentzel et al., 1966) and (c) diffusion approximated by Fick's law. The assumptions involved in this rationale and the equations needed have been previously discussed (e. g. Jacobs (1952), Tedeschi, 1959).

The photometric method used to measure mitochondrial osmotically active relative volume (Tedeschi and Harris, 1958) presents a simple and direct method of following rapid changes in mitochondrial volume as long as the refractive index of all solutions (calibrations and experimental) is kept the same. The reciprocal of the optical density is directly proportional to the mitochondrial osmotically active volume.

b. Procedures

The isolation of mitochondria was carried out as previously described (Tedeschi, 1959). The details of the photometric method have been previously presented (Tedeschi and Harris, 1958). The changes in transmitted light were recorded with a Beckman linear-log recorder. The light source and the photocell of a Coleman Jr. spectrophotometer were used at a wavelength of 580 mμ. Each 0.10 ml sample of mitochondrial stock suspension in 0.3 molal sucrose and 0.01 M tris, pH 7.4, was diluted to 1.10 ml with the same medium. 0.05 ml containing 2,4 dinitrophenol (DNP) in 0.01 M tris were added to

bring the DNP concentration to 0.1 mM. These suspensions were incubated for 5 minutes at 31 ± 1°C. After this incubation 3 ml of either 0.3 molal malonamide or 0.137 molal Na acetate, 0.0697 molal sucrose, 0.01 M tris containing 0.1 mM DNP were added. The time of addition of the penetrants corresponds to the 0 time of penetration indicated in the figure. Penetrant and calibration solutions were maintained at 24 ± 2°C. During the determinations the spectrophotometer was cooled by circulating air. At the conclusion of the determinations the suspensions were 2 ± 1°C warmer than the original penetrant solutions. To avoid any systematic error introduced by deteriorations of the mitochondria the order of the control, experimental and calibration determinations were randomized throughout the experiment. The controls were subject to identical procedures but in the absence of DNP. The volumes reported correspond to osmotically active volume where the mitochondrial volume in 0.336 osmolal of a non-penetrant is taken arbitrarily as unity (see Tedeschi, 1958).

RESULTS

Previous work (Tedeschi (1959)) indicates that the penetration of several non-electrolytes (low molecular weight, relatively lipid soluble) remains unaffected by swelling. The kinetics are consistent with no change in either permeability or the surface area available for penetration. Accordingly, the kinetics of the volume change follow the pattern expressed in equation (1):

$$(1) \qquad V^2 = V_o^2 + 2PAV_o t$$

where V is the osmotically active volume at any time (t), V_o is the initial volume, P the permeability constant and A the surface area (see Jacobs, 1952). A plot of V^2 vs t provides a slope ($2PAV_o$) which is an indication of permeability (see Fig. 1A). Equation (1) makes the assumption that no significant amounts of a non-penetrant are present externally. Since there is a considerable concentration of sucrose in the final medium, Equation (2) which does not involve this assumption should be used.

$$(2) \quad P\,A\,t = \frac{(C_o + C_s)}{C_o^2} \left[C_o\,(V-V_o) - C_i V_o \ln \left(\frac{C_i V_o + C_o V}{C_i V_o + C_o V} \right) \right] = f\,(V)$$

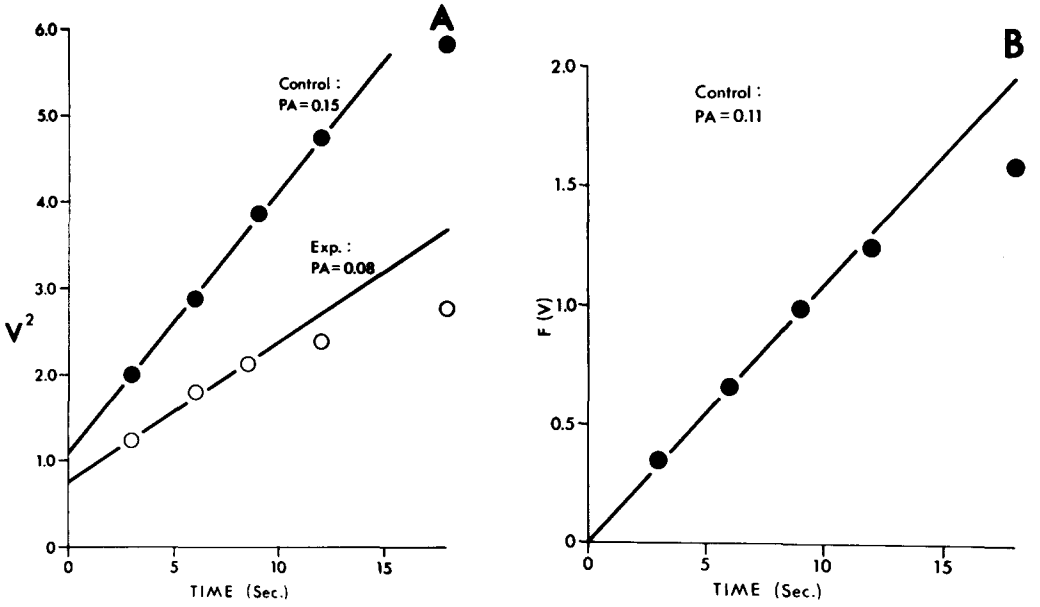

Fig. 1. Kinetics of swelling of a typical experiment. V is expressed in relative units (see text).

A. Malonamide penetration, experimental and control. V^2 is time. The standard deviation of each V is as follows:

time (sec)	3	6	9	12	18
Exp.	0.09	0.08	0.06	0.08	0.07
Control	0.07	0.07	0.06	0.07	0.07

B. Malonamide penetration (control). The function of equation (2), F (V) is time.

An analogous equation has been previously presented by Schiodt (1933). In equation (2), C_o is the external concentration of non-penetrant, C_s the concentration of penetrant and $C_i = C_o + C_s$. A plot of f (V) vs time provides a slope which corresponds to PA (Fig. 1B). In practice equation (2) presents a number of practical problems since it depends entirely on V_o which cannot be obtained directly.

In the initial phase of the swelling (V lower than 2), either equation (1) or (2) provides approximately the same answer (Table 1). Although the apparent penetrability (PA, see below) is somewhat higher with equation (1), this equation was used nevertheless because of its simplicity. From equation

TABLE 1

Comparison of the use of equation (1) with equation (2) for malonamide penetration. 4 independent experiments; the deviations are standard deviations. PA is defined in the text.

	Equation (1)	Equation (2)
PA	0.16 ± 0.03	0.11 ± 0.02

(1) it is clear that a plot of V^2 as a function of time should result in a straight line. The slope would be $2PAV_o$ and the intercept V_o^2. This is illustrated in Fig. 1A. P can be obtained by substituting the appropriate values for V_o and A (see Tedeschi, 1959). To avoid the use of arbitrary assumptions, in particular in specifying A, these parameters were left in relative units and the results (e. g. Table 2) are expressed as PA (defined for purposes of this paper as penetrability), V_o and V_{eq} (the volume after 120 seconds).* In this paper, the osmotically active volume of mitochondria in .336 osmolal solution of a non-penetrant has been taken as unity; A, V_o and PA given are relative to this value. The equilibrium volume is useful since it gives information as to whether osmotically active internal solutes have leaked out during the procedures. It is difficult to decide precisely when equilibrium has occurred. For this reason, the volume at 120 seconds was taken arbitrarily as V_{eq} (Table 2). The data are expressed in terms of PA, V_o

TABLE 2

Kinetic parameters in the presence and absence of DNP (see Equation (1)) and text. Mean of four independent experiments. The deviations are standard deviations.

Penetrant			PA	V_o	V_{eq}
Malonamide	a.	Control	0.16±0.03	0.85±0.20	2.76±0.19
	b.	Exp.	0.11±0.02	0.74±0.16	2.02±0.16
Na acetate	a.	Control	0.08±0.03	0.74±0.16	2.60±0.11
	b.	Exp.	0.05±0.01	0.61±0.04	1.58±0.10

*with the assumptions previously used P~1.4 X 10^{-3} cm/hr for malonamide

and V_{eq} in Table 2. Two penetrants were chosen to test whether a change in permeability occurs. One is malonamide, the other Na acetate. These were chosen since their penetration was known to be slow enough to be followed with the simple system used.

In all the experiments (Fig. 1A and Table 2) it is clear that the rate of swelling is lower in the samples incubated with DNP. The results with the bimolecular phospholipid system report a decrease in electrical resistance of 99.6% from the control value. The apparent changes in permeability encountered by this study are smaller, and in the opposite direction. The apparent penetrability is decreased by about 30-40%. However, the final volume (V_{eq}) is also decreased by 25-40%. Therefore this difference is not likely to represent a change in permeability but rather the result of a concomitant shrinkage possibly brought about by the leakage of internal ions. The loss of K^+ in the presence of DNP is well documented in a number of experiments (e. g. Stanbury and Mudge (1953) and Berger (1957)).

CONCLUSIONS

The results obtained with both malonamide and sodium acetate are not consistent with the concept of a general increase in the permeability of mitochondria in the presence of 2,4 dinitrophenol.

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