OSMOTIC REVERSAL OF Ca^{2+} INDUCED MITOCHONDRIAL SWELLING

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Isolated mitochondria from a number of sources are known to swell in the presence of Ca^{2+} (e. g. Cleland and Slater (1953), Raaflaub (1955), Chapell et al (1963), Saris (1963)). Under at least some of these conditions, a pronounced uptake of Ca^{2+} also occurs (e. g. Slater and Cleland (1953), Chapell et al (1963), Saris (1963)).

A number of arguments have been previously presented for the hypothesis that mitochondrial swelling is generally osmotic in nature (Tedeschi (1961)). In this light, the effect of Ca^{2+} could be regarded as an increase in the permeability of the mitochondria with consequent penetration of the suspending solute and hence swelling. This, in fact, has been previously proposed (Cleland and Slater (1953), Saris (1963)). Another alternative may also come to mind. It has been shown by a number of workers that divalent cations such as Ca^{2+}, Mg^{2+}, Mn^{2+} and Sr^{2+} can be translocated into mitochondria isolated from a variety of tissues (e. g. De Luca and Engstrom (1961), Vasington and Murphy (1962), Brierley et al (1962), Rossi and Lehninger (1963), Chapell et al (1963), Carafoli (1965) Hodges and Hanson (1965)). The transfer depends on oxidative metabolism or ATP hydrolysis and it is blocked by a number of metabolic inhibitors. Under the conditions used in most works, the transfer of cations has been found accompanied by that of inorganic phosphate with consequent precipitation of the salt.

However, at least for a limited uptake, the presence of phosphate is not an absolute requirement. (Slater and Cleland (1953), De Luca and Engstrom (1961), Saris (1963) and Carafoli (1965)). Therefore, it is conceivable that at least some of the swelling could be brought about by the translocation of Ca^{2+} and an accompanying anion, in an osmotically active form. However, the swelling observed in some experiments cannot be the result of such translocation since (a) the Ca^{2+} taken up is of an apparent intramitochondrial concentration of approximately 0.02 to 0.04 M (Slater and Cleland (1953), Saris (1963)) and is therefore insufficient to maintain the extensive swelling observed, and, (b) in some of the experiments (Saris (1963), Tedeschi and Hegarty, unpublished), the uptake precedes the swelling in time.

Regardless of mechanism, an osmotic swelling should be quantitatively osmotically reversible. The present experiments were designed to examine this possibility.

METHODS

Rat liver mitochondria were isolated from rats of the Holtzman strain weighing 300-400 gm, which had been starved approximately 16 hours. The method has been previously presented (Tedeschi (1961)). The livers were homogenized in 0.25 M sucrose (pH 6-8). After removal of the large particles by centrifugation at 600 g for 15 minutes, the mitochondria were isolated by centrifugation at 8.500 g for 15 minutes. The materials were maintained between 0 and 4°C. The mitochondria were generally resuspended in 0.3 molal sucrose, 0.01 M tris (trimethylol amino methane), pH 7.4 or in the case of Exp. 4, Table II, in 0.25 M sucrose. They were then stored at 0°C.

The photometric procedures used have been previously described

(Tedeschi and Harris (1958)). A wavelength of 520 mμ was used. The experimental suspensions were maintained at 23± 1°C. The mitochondrial volumes reported in this study correspond to the apparent relative osmotically active volumes. The osmotically active volume in 0.335 osmolal solutions of non-penetrant has been taken arbitrarily as unity.

RESULTS AND DISCUSSION

In typical experiments, mitochondria swell only slightly when incubated in the absence of Ca^{2+}. Such a case is represented in Fig. 1, Curve 2. In this case, 0.1 cc of the mitochondrial stock suspension was added at zero time to 3 cc of 0.3 molal sucrose, 0.01 M tris, 0.01 M Na succinate, 10^{-5} M Na_2HPO_4, 10^{-5} $MgCl_2$, 0.003 M Na ATP, at pH 7.4.

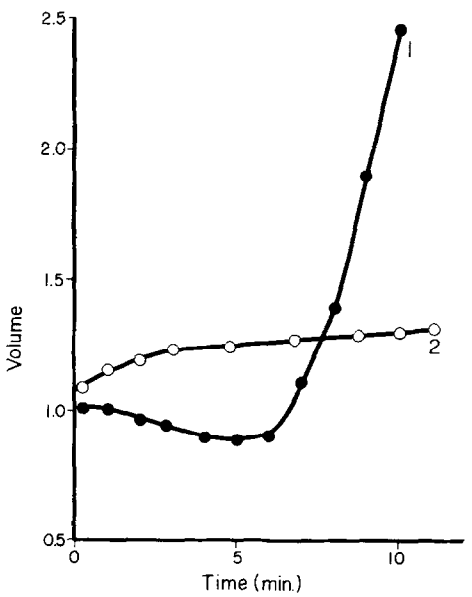

Fig. 1. Ca^{++} induced mitochondrial swelling, see text. Means of 5 determinations.
The standard errors are approximately the diameter of the circles or smaller.
Curve 1: Mitochondrial swelling in the presence of Ca^{++}.
Curve 2: Mitochondrial swelling in the absence of Ca^{++}.

The presence 10^{-4}M $CaCl_2$ in the same medium, after a lag period, induces a greater swelling (Fig. 1, Curve 1). A swelling such as this continues without change after the addition of 0.6 cc of the same medium followed by mixing (Fig. 2, Curve 1). On the other hand, the addition of 1.58 molal NaCl containing the same chemical complement, except for the absence of sucrose, results in an immediate reversal (Fig. 2, Curve 2) The extent of reversal is approximately that predictable from osmotic considerations, shown by the horizontal dashed line (see Tedeschi and Harris (1958)). Two similar experiments are shown in Table I.

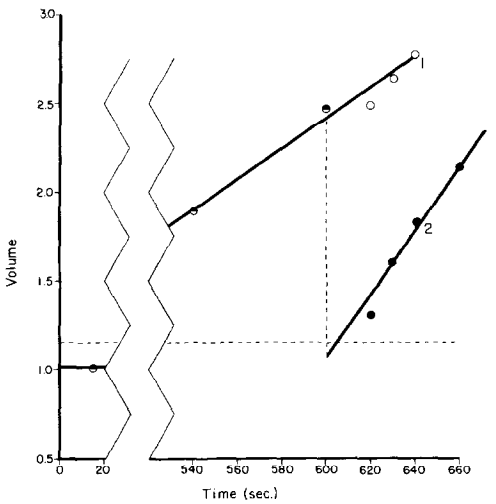

Fig. 2. Reversal of Ca^{++} induced mitochondrial swelling. This experiment was carried out simultaneously to that of Fig. 1 and with the same preparation. Means of 5 determinations. The standard errors are approximately the diameter of the circles or smaller.
Curve 1: Control
Curve 2: Osmotic reversal

After the reversal, an apparent swelling follows. A similar rapid swelling has been shown to occur in the case of the reversal of the osmotic swelling following exposure to dilute raffinose solutions. Therefore, it is possible that this secondary swelling might not be the

TABLE I

Reversal of Ca^{++} induced swelling. V_t is the volume preceding the addition of reversal solutions by about 1-2 seconds. V at 20 seconds, is the volume 20 seconds after the addition of the reversal solution and V_r is an estimate of the volume obtained by extrapolation, corresponding to the time of addition of the reversal solution. All the volumes are expressed as apparent relative osmotically active volumes (see Methods). t is the time of reversal in minutes. The errors are standard errors. The conditions of the experiments are the same than those of Fig. 1 except that in Experiment 1, the $MgCl_2$ concentration is 10^{-3} M.

Exp.	n. of determinations	t	V_t	V at 20 sec.	V_r	theoretical V
1	4	9	1.48±0.02	0.97±.11	0.65	0.69
2	5	10	1.62±0.07	0.79±.04	0.56	0.76

result of the exposure to the Ca^{2+}, but an independent phenomenon.

The apparent shrinkage which precedes the swelling has been previously observed (Saris (1963)). It may be of considerable interest, since during this period the maximum uptake of Ca^{2+} seems to be taking place.

The conditions necessary for the reversal have been examined to some extent. Neither the swelling nor the reversal require the presence of succinate (Table II, Exp. 3 and two experiments which are not shown). This is not surprising since mitochondria isolated rapidly from rat liver seem to have an endogenous substrate. The swelling does not require the trace of phosphate present in the other experiments (Table II, Exp. 3a and 3b), and, in fact, neither does the reversal (Table II, Exp. 3c). However, ATP at high concentrations seems to be necessary for reversal (Table II, Exp. 1a and 2a). This experiment supports the proposal of others that ATP, frequently necessary during oxidatively supported Ca^{2+} accumulation, may have the indirect role of maintaining mitochondrial structure.

The conditions of Exp. 4 are of some interest. The medium (see legend of Table II) is capable of supporting Ca^{2+} accumulation without the presence of either phosphate or ATP as reported by Rossi and Lehninger (1964). A good portion of the swelling, but not all of it, is osmotically reversible.

TABLE II

The symbols are the same as those of Table I. The conditions of Exp. 1 to 3 are as presented in the text, except for the absence of Na succinate and the change specified. In Exp. 4 the conditions are as follows: 0.02 M tris, 0.08 M NaCl, 0.01 M $MgCl_2$, 0.01 M Na succinate, and 1.5×10^{-4}M $CaCl_2$, pH 7.2. Where reversal was not carried out, the suspensions were appropriately diluted and mixed. Exp. 1 and 2 were carried out on the same preparation.

Exp.	Conditions	n. det.	t	V_t	V at 15 sec.	V_r	Theoret. V
1. a.	ATP=10^{-4}	4	6	1.96±0.08	2.11±0.11	—	0.82
b.	no Ca^{2+} ATP=10^{-4}	3	6	0.92			
2. a.	ADP=10^{-4} ATP=0	4	10	1.96±0.20	2.16±0.14	—	0.82
b.	no Ca^{2+} ADP=10^{-4} ATP=0	3	10	1.12			
3. a.	no Pi_{2+}	4	5	1.56±0.15	0.96±0.07	0.57	0.65
b.	no Ca, noPi	4	5	1.00±0.06			
c.	no reversal	4	5	1.39±0.08	1.49±0.13		1.39
4. a.	see legend	4	9	3.05±0.20	2.23±0.15	1.58	0.99
b.	no Ca^{2+}	4	9	1.93±0.13			
c.	no reversal	4	9	2.91±0.08	3.00±0.03		2.91

This result is not easily interpreted, since the meaning is somewhat obscured by the fact that considerable swelling also seems to occur in the absence of Ca^{2+}.

CONCLUSION

The demonstration of the osmotic reversibility of Ca^{2+}- induced swelling is consistent with the possibility that the underlying phenomenon

is osmotic in nature. The results do not necessarily exclude alternative mechanisms. However, alternative hypotheses must be capable of explaining the osmotic reversibility. This work was supported by grants from the American Cancer Society, Inc. (P-183) and USPHS (GM-09156).

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