PHOSPHATE CARRIER OF RAT-LIVER MITOCHONDRIA: ITS ROLE IN PHOSPHATE OUTFLOW

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It has been reported recently that inhibition of oxidative phosphorylation by some organic mercurial compounds was due to inhibition of phosphate entry into the mitochondria (Tyler, 1968; Fonyo, 1967; Fonyo and Bessman, 1968). Chappell and Crofts (1965) postulated earlier that phosphate penetrated the mitochondrial membrane via a carrier system. The possibility was therefore raised that the mercurials interacted with the phosphate carrier. In this paper evidence is presented that the outflow of phosphate from mitochondria is mediated by the same mercurial-sensitive system that permits phosphate entry.

Methods. Rat liver mitochondria were isolated from a medium containing 0.25 M sucrose and 1 mM EDTA and washed twice in EDTA-free sucrose. Mitochondrial swelling and shrinkage were measured by recording changes in the apparent optical density of mitochondrial suspensions in a spectrophotometer set at 520 mμ using a high-absorbance standard in the reference path. When the inorganic phosphate content of the mitochondria was determined, the incubation was carried out in plastic centrifuge tubes which were centrifuged in the N° 69182 rotor of the MSE-17 refrigerated centrifuge. The rotor was accelerated to 14 000 rev per min and then stopped, a procedure lasting 12 minutes. After decanting the supernatant, the tubes were drained by inversion, wiped dry and the mitochondrial sediment was extracted with cold 5 per cent perchloric acid. The inorganic phosphate content of the extract was determined according to Lohmann and Jendrassik (1926). No attempt was made to correct the data for the extra-

mitochondrial suspending medium that was trapped between the particles.

Results and discussion. Swelling during gramicidin-induced K^+-ion accumulation depends on the presence of a permeating anion and is reversed immediately on abolishing the energy supply (Chappell and Crofts, 1965). On this basis, the shrinkage elicited by the addition of antimycin A to swollen mitochondria was used as an indicator of the anion outflow. The time course of volume changes induced by gramicidin is illustrated in Fig. 1. The addition of antimycin A after swelling induced by gramicidin resulted in an increase of apparent optical density and the return of the latter to a slightly higher level than was recorded initially, indicating that the shrinkage was complete (Fig. 1a). The mitochondrial volume did not return to the initial value if p-mercuribenzoate was added during swelling in a phosphate medium, and the mitochondria remained in the swollen state during the entire period of observation (Fig. 1b). There was a slight increase in absorbance following addition of the mercurial and another slight increase after antimycin A. Shrinkage in swollen, mercurial-treated mitochondria was however reestablished by added thiols, e.g. 2-mercaptoethylamine (Fig. 1d). The mercurial had no effect on mitochondrial shrinkage in a medium where acetate was the only permeant anion present (Fig. 1c). In media which contained acetate and phosphate, the addition of p-mercuribenzoate during gramicidin induced swelling inhibited the shrinkage phase.

In these experiments it was essential to include Mg^{++}-ions in the medium. If Mg^{++}-ions were absent, addition of p-mercuribenzoate to swollen mitochondria resulted in considerable shrinkage even before antimycin A had been added. In the presence of 5 mM Mg^{++} however, only a moderate degree of shrinkage was induced by the mercurial, as seen in Fig. 1.

The mercurial interfered with mitochondrial shrinkage if the mitochondria had accumulated phosphate, whereas it was ineffective if only acetate was accumulated in the mitochondria. These results suggested that the inhibition of mitochondrial shrinkage by the mercurial was due to the prevention of phosphate outflow, and this conclusion was confirmed by direct measurements

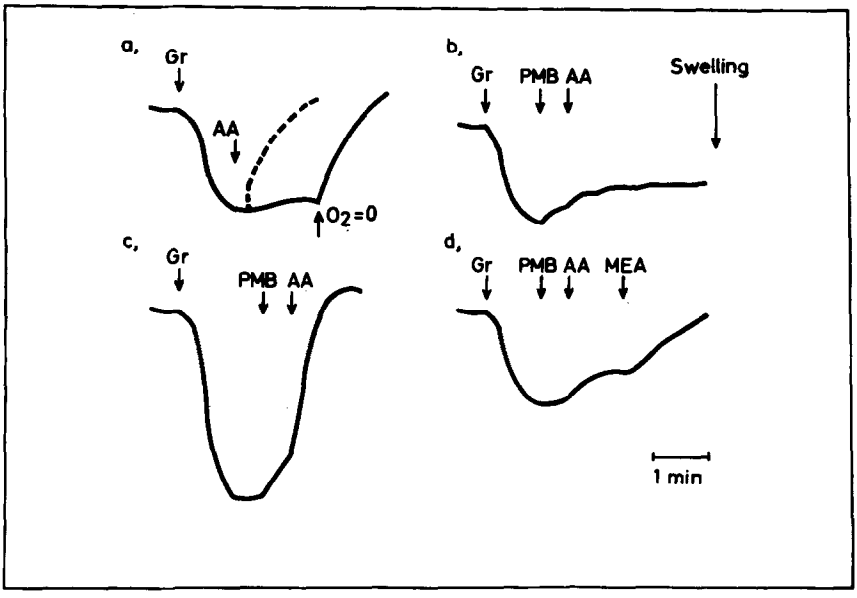

Fig. 1. Effect of p-mercuribenzoate on mitochondrial volume changes induced by gramicidin and antimycin A. Mitochondria were incubated in the presence of 175 mM sucrose, 20 mM KCl, 25 mM tris-Cl, 10 mM tris-succinate, 5 mM $MgCl_2$ and 1 uM rotenone: the pH was 7.4. In a, b and d 3.3 mM tris-phosphate and in c 6.6 mM tris-acetate was present. Mitochondrial protein: 1.7 mg per ml. Abbreviations: Gr: gramicidin D 1 ug per ml; AA: antimycin A 1 ug per ml; PMB: p-mercuribenzoate 46 uM; MEA: 2-mercaptoethylamine 133 uM. In trace a the solid line indicates spontaneous shrinkage of the mitochondria after exhaustion of the dissolved oxygen, while the broken line indicates shrinkage on addition of antimycin A. The temperature was 25°.

of the phosphate content of the mitochondria (Table I). Mitochondria were incubated aerobically with succinate as oxidizable substrate and ion uptake was induced by gramicidin. Subsequent addition of antimycin A led to loss of the accumulated salt, since the phosphate content of the pellet was low. If p-mercuribenzoate was added after gramicidin but before antimycin A, the phosphate content of the mitochondria was significantly higher. Any other sequence of additions, e.g. if the mercurial was added before the gramicidin or after antimycin A, resulted in a lower phosphate content. Although the inorganic phosphate retained in the mitochondria was some 20 per cent lower in the absence of Mg^{++} than in its presence, it was evident that the mercurial

TABLE I

Inorganic phosphate content of mitochondria following incubation with gramicidin, antimycin A and p-mercuribenzoate

Sequence of additions	Phosphate /umoles/g protein
Gramicidin, antimycin A	15.7
p-Mercuribenzoate, gramicidin, antimycin A	21.1
Gramicidin, antimycin A, p-mercuribenzoate	16.9
Gramicidin, p-mercuribenzoate, antimycin A	46.1

Mitochondria were incubated in the presence of 200 mM sucrose, 20 mM KCl, 1.3 mM tris-phosphate, 3.3 mM tris-succinate, 5 mM $MgCl_2$ and 1 /uM rotenone. 3 ml medium contained 9.7 mg mitochondrial protein. 5 /ug gramicidin D was added at 1 min and 5 /ug antimycin A at 3 min; the total incubation time was 5 min. p-Mercuribenzoate, if added, was 40 /uM. Temperature was 26°.

blocked phosphate outflow also in the Mg^{++}-free medium. In this case the apparent optical density did not parallel the ion content of the mitochondria, but the reason for this discrepancy is not yet clear.

The data of Fig. 1 and Table I indicate that the mitochondria contain a mercurial-sensitive carrier that mediates phosphate outflow, and as phosphate entry into mitochondria is also blocked by mercurials the same carrier seems to be involved in both movements. Similar observations and conclusions have been made by Dr D.D. Tyler (personal communication).

Following mercurial treatment the mitochondria retained K^+-ions together with phosphate (Fonyo, unpublished observations). Cation outflow was evidently limited either by a developing membrane potential or by an electro-chemical concentration gradient. The role of ionic concentration gradients and of a membrane potential in energy conservation reactions of mitochondria has been discussed recently (Mitchell, 1966; Cockrell, Harris and Pressman, 1967; Glynn, 1967). The inhibition of ion-fluxes by

mercurials may be of future use in the analysis of the energy conservation sequence.

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