STRUCTURAL CHANGES IN ISOLATED RAT KIDNEY MITOCHONDRIA TREATED WITH PHLORIZIN AND ATP (1)

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The optical density and chemical properties of isolated mitochondria under normal and experimental conditions have been the subject of numerous investigations (see Lehninger, 1962; Lotspeich, 1961; Novikoff, 1961 for reviews), but their morphological changes during swelling and contraction have been studied less exhaustively. It has been generally accepted that swelling and contraction are mainly related to the permeability properties of the mitochondrial membranes and that such properties are connected with the energy metabolism (Lehninger, 1962).

To understand better the morphological changes which accompany volume changes, we have isolated rat kidney cortex mitochondria according to Tapley (1956).

One half of the mitochondrial pellet was resuspended in 0.5 M sucrose. Aliquots of the mitochondrial suspension, immediately after isolation, were placed in the following media at 20°C: 0.5 M sucrose (control); 0.5 M sucrose plus $3x10^{-3}$ M phlorizin; 0.5 M sucrose plus $3x10^{-3}$ M phlorizin plus $5x10^{-3}$ M ATP. The mitochondria were added in sufficient amounts to give an initial optical density near 0.5. After thorough mixing, such systems were read every minute during 25 minutes at 520 mu in a Beckman B spectrophotometer (2).

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Fig. 1 shows a typical set of curves. Phlorizin gives a steadily declining curve. Phlorizin plus ATP, on the other hand, not only prevents the phlorizin-induced swelling but causes an increase in optical density. The control gives constant readings during the whole 25 minutes.

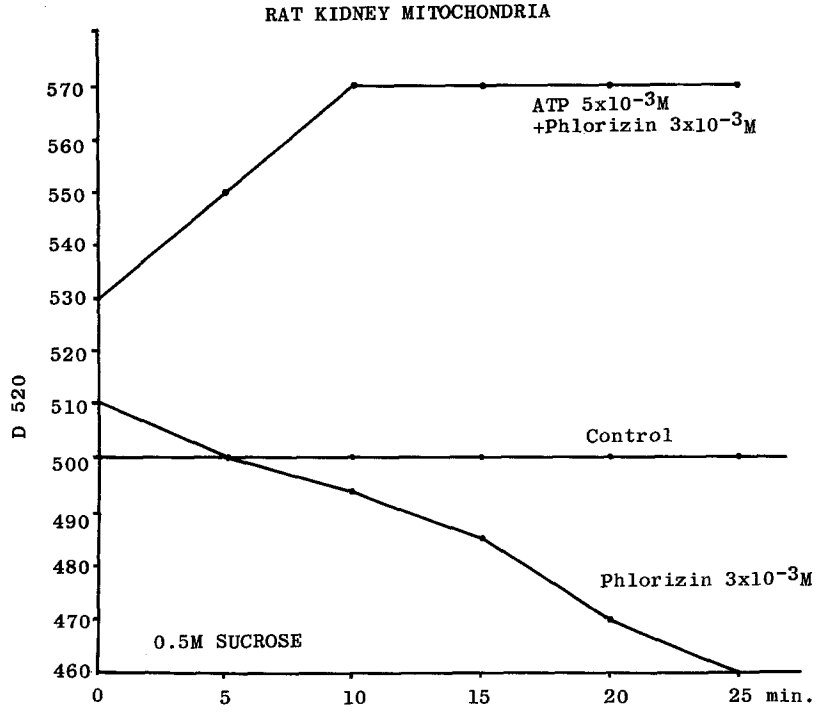

Figure 1

The other half of the mitochondrial pellet was incubated in the same media for 15 minutes, fixed in 1% buffered OsO_4 for 2 hours and embedded in epoxy. Ultrathin sections were stained with lead citrate and uranyl acetate and studied in a Siemens Elmiskop I and an RCA EMU-3D electron microscopes.

The mitochondrial volumes were calculated from the average of three diameters of the mitochondria as shown by the electron micrographs, considering the mitochondria as spheres (fig. 2).

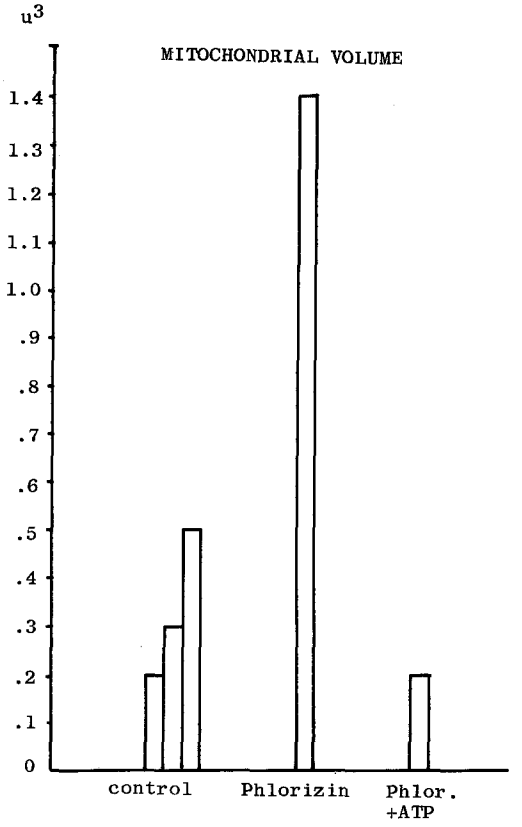

Figure 2

Microdensitometric measurements of the electron micrographs were done using a selenium photocell-galvanometer attached to a light microscope. Readings are given in relative values (fig. 3).

The control pellet contains three types of mitochondria in approximately equal amounts: a) medium, volume 0.3 u^3, relative density 25; b) small and dark, 0.2 u^3, relative density 50; c) large and pale, 0.5 u^3, relative density 15. These three types of mitochondria are, in our opinion, different dynamic stages of the normal mitochondrion, i.e.,the same mitochondrion adopts any of the three stages according to functional requirements.

Addition of phlorizin to the mitochondrial suspension causes the polymorphism to change into uniformity; almost all the mitochondria change into the large and pale stage, averaging 1.4 u^3 and relative density 10. The internal compartment

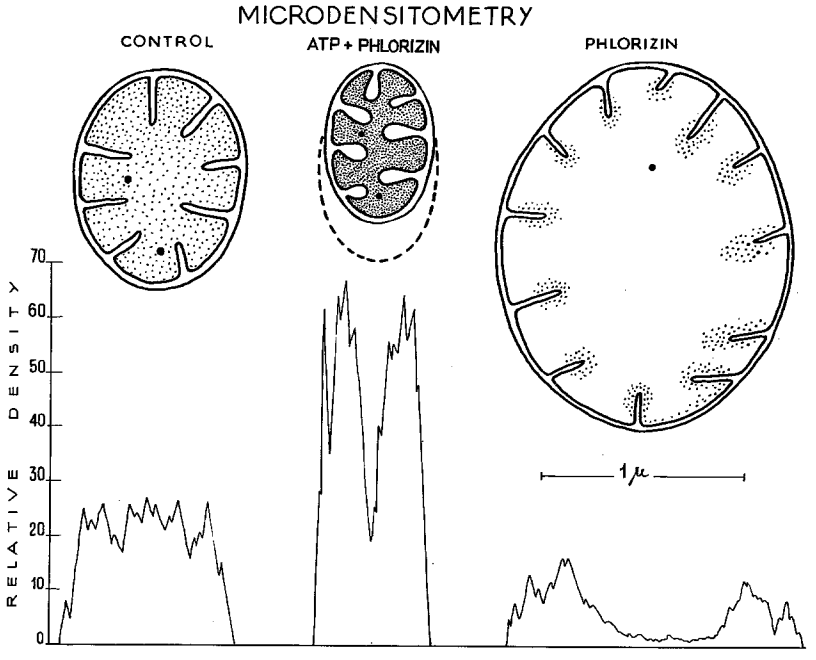

Figure 3

is the one mainly involved in such swelling; the outer compartment decreases in thickness by virtue of outward pressure exerted from the swollen inner compartment.

On the other hand, ATP, when added simultaneously with phlorizin, causes the mitochondria to become small and dark; they average 0.25 u^3 and relative density 65. The affinity of the matrix for uranyl is the strongest found in this work. The cristal lumens appear swollen; the outer membrane adheres closely to the inner membrane at some points and is partially or completely detached from it at other places of the mitochondrial contour. At the points where the outer membrane is broken, the mitochondrion exhibits disorganization of its structure.

On the basis of these findings, we postulate that:

1) The inner compartment of the mitochondrion is the one chiefly involved in swelling and contraction.

2) Shrinking is probably related to the following mechanism: after the ATP concentration in the matrix increases, a contract-

ile protein extrudes substances into the outer compartment. This contraction causes the decrease in volume of the matrix and the swelling of the cristal lumens.

3) Phlorizin-induced swelling can be ascribed to blockage of ATP resynthesis followed by increase of water content in the matrix.

4) Critical ATP levels act as a trigger of swelling and contraction of the matrix, playing a role in the regulation of the passage of materials in and out of the mitochondrion.

5) The continuity of the outer membrane is a guaranty for the structural integrity of the mitochondrion even during the most extreme volume changes.

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