SOME OBSERVATIONS ON THE SWELLING OF MITOCHONDRIA CAUSED BY NATURALLY-OCCURRING AGENTS *

M. A. Spirtes

Department of Pharmacology, Hahnemann Medical College, Philadelphia, Pennsylvania

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Recently, a number of reports have appeared describing the effects of preparations obtained from Tetrahymena pyriformis (Eichel, 1959; 1960), rat liver mitochondria (Lehninger and Remmert, 1959), blow-fly thoraces (Lewis and Fowler, 1960), wax-moth larvae (Wojtczak and Wojtczak, 1960), and mitochrome (Hülsmann, Elliot and Slater, 1960) on electron-transport mechanisms, oxidative phosphorylation, ATPase activation, ATP-Pi interchange and mitochondrial permeability. All but one of these preparations have been shown to contain extractable fatty acids which are active on one or more of the metabolic parameters mentioned above. The active component of the Tetrahymena preparations can be formed enzymatically by treating cell-free Tetrahymena homogenates with crystalline lecithinase A and therefore is also probably lipid in nature. Lehninger & Remmert (1959) postulated that the lipid extract from rat liver mitochondria might be concerned with the in vivo control of mitochondrial membrane permeability since it lowered the optical density (O.D.) of intact liver mitochondria suspended in 0.125 M KCl.

In the course of testing the effect of chlorpromazine on mitochondrial permeability in this laboratory, various swelling agents were added to rat brain and liver mitochondria. Among these were Eichel's Tetrahymena preparation, rat liver mitochondrial swelling agent and an

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analogous factor extracted from rat brain mitochondria. None of these preparations lowered the O.D. of suspensions of intact rat brain mitochondria, although each decreased the O.D. of intact rat liver mitochondria. The fact that brain mitochondria do not respond to the swelling agents tends to weaken the general concept that such agents are physiological regulators of mitochondrial permeability.

Liver mitochondria were prepared according to Hogeboom and Schneider (1948) and brain mitochondria as directed by Brody and Bain (1952). Liver mitochondrial swelling factor was prepared according to Lehninger (1959); the brain mitochondrial swelling factor was prepared in a similar manner. When ready to be used in the O.D. studies, the solvent portion of the mitochondrial swelling factor suspensions was replaced with an equal volume of the solution in which the intact mitochondria were later to be suspended (Lehninger, 1959). 0.3 ml. of this swelling agent suspension in a total volume of 3 ml. was used to test for mitochondrial swelling. The Tetrahymena particulate preparations were made according to Eichel (1960). 0.1 - 0.2 ml. of these preparations was added to each cuvette containing a total volume of 3 ml. The contents of all cuvettes were kept at pH 7.4 by the addition of Tris buffer adjusted to a final concentration of 0.02 M. The fall in O.D. at 520 mu (Beckman DU spectrophotometer) was recorded beginning with the moment when the mitochondria were added to the rest of the components in the cuvette.

Although rat brain mitochondria reacted fairly uniformly when prepared fresh daily, this was not true of liver mitochondria. The latter, especially when suspended in sucrose, varied greatly in endogenous swelling rate — from a fall of a few O.D. units (1 unit = .001 on the density scale) to 200 units. In addition, the swelling capacity frequently increased when the mitochondria were kept at 0° C for 1-2 hours. As the control swelling rate approached 200 units, addition of swelling factors produced no additional drop in O.D. The mitochondria

were therefore suspended in the lowest concentration of sucrose causing an endogenous swelling rate of less than 100 units in 10 minutes. It was also observed that the O.D. of mitochondria in isotonic KCl usually dropped at a greater rate than that of particles suspended in isotonic sucrose. This was true for both brain and liver mitochondria (Table 1).

TABLE 1

Effect of Swelling Factor from Rat Liver Mitochondria on Intact Rat Brain or Liver Mitochondria as Measured by Changes in Optical Density

Mitochondria	Suspension Medium	Average change in O.D. units in 10 minutes	
		Mitochondria alone	Mitochondria + Liver factor
Brain	Sucrose-Tris	+6	+4
	KCl-Tris	-75	-78
Liver	Sucrose-Tris	-12	-84
	KCl-Tris	-43	-138

The suspension media consisted of 0.25-0.37 Sucrose + 0.02 Tris buffer pH 7.4. Mitochondria from 2 G. wet wt. of brain or 1 G. of liver were made up to 1 ml. with 0.25 Molar sucrose. 0.1-0.2 ml. of liver mitochondrial swelling factor or 0.3 ml. of brain mitochondrial swelling factor was suspended in the same medium used for the intact mitochondria. To this, excess suspension medium was added to make a total volume of 3 ml. with 0.06 ml. of liver mitochondria or 0.1-0.2 ml. of brain mitochondria. Initial O.D. measured on the Beckman DU spectrophotometer should lie between 0.4 - 0.6. Spectrophotometric readings taken at 520 mu wave length. Mitochondrial exposure time to swelling factor was 10 minutes. Six to 20 experiments of each type were performed.

Table 1 indicates that liver mitochondrial swelling factor does not cause any additional decrease in O.D. of rat brain mitochondria whether the latter are suspended in a sucrose-Tris or KCl-Tris buffer. The same swelling factor does, however, cause an additional O.D. fall of liver mitochondrial suspensions in either medium.

Since it was possible that the mitochondria from each organ responded only to a mitochondrial swelling factor from the same organ, rat brain

swelling factor was next added to brain and to liver mitochondria. As in the case of the liver factor, the brain factor did not increase the swelling rate of intact brain mitochondrial suspensions but, when added to intact rat liver mitochondria, increased the O.D. fall more than three-fold.

Eichel's inhibitor of electron-transport and phosphorylation also caused a marked, added decrease in O.D. of rat liver mitochondria suspended in either KCl or sucrose media, but like the mammalian swelling factors, it had no effect on brain mitochondria. The extent of the added drop in O.D. of the liver particles over the endogenous decline was the same whether the mitochondria were suspended in KCl or sucrose. The fact that the Tetrahymena inhibitor factor, like the Lehninger swelling factor, promoted liver but not brain mitochondrial swelling is further evidence for a strong similarity between them.

Since brain, spleen, ovary and testes mitochondria are not known to respond to thyroxin addition by increases in metabolism or swelling, the effect of the three swelling agents used in these experiments is being investigated on all the above types of mitochondria. These projected experiments should provide further evidence for or against the hypothesis that the mitochondrial swelling factors have general physiological significance, particularly if factors from all the organs tested are prepared and tried on intact mitochondria from the same organs.

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