The Electrophoretic Behaviour of Mitochondria from Kidney and Liver of Rats

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1. The electrophoretic mobility values for mitochondria prepared from rat kidney and liver measured in 0.125M-potassium chloride-0.02M-tris, pH 7.4, are: $-0.78(\text{s.e.m.} \pm 0.02)\mu/\text{sec.}/\text{v}/\text{cm.}$ (29 experiments) and $-1.06(\text{s.e.m.} \pm 0.01)\mu/\text{sec.}/\text{v}/\text{cm.}$ (21 experiments) respectively. 2. These mobility values are unaffected by washing and spontaneous swelling at 25°, indicating a stable electrokinetic surface. 3. The mobility of rat-kidney mitochondria is unaffected by thyroxine-induced swelling, or by the state of hydration of the rat. 4. pH-mobility curves show similar surface ionogenic groups for kidney and liver mitochondria; their isoelectric points are pH 3.9 and pH 4.4 respectively.

It is well known that the membranes of the mitochondrion contain the enzymes associated with the important processes of electron transport and oxidative phosphorylation. Therefore these membranes have been the subject of extensive investigations. In particular the structure and the properties of contractility and permeability have been widely studied (Lehninger, 1962). Although many reagents cause the swelling of isolated mitochondria, the magnitude of the swelling depends on the tissue from which the mitochondria are prepared (Tapley & Cooper, 1956; Spirtes, 1961). For example, Greenbaum & Dicker (1963) showed that the extent of swelling caused by the neurohypophyseal hormones depended on whether the mitochondria were prepared from liver, kidney cortex or kidney medulla. Since the ionogenic groups might be involved in the binding of hormones to the membrane it was decided to investigate the surface of kidney and liver mitochondria, by using the technique of micro-electrophoresis. This technique has been used frequently to study the nature of the surface components of many biological particles (James, 1957; Brinton & Lauffer, 1959), but mitochondria have not been studied in any detail (Douglas, Laycock & Boulter, 1963). A preliminary account of this work has been presented at a meeting of The Biochemical Society (Plummer, 1964).

EXPERIMENTAL

Materials. All solutions were prepared in water that had been deionized (The Permutit Co. Ltd., London, W. 4), then distilled from a Pyrex still (L.V.D. Scorah, Birmingham).

The tris, obtained as a highly purified preparation (British Drug Houses Ltd., Poole, Dorset), was recrystallized twice in distilled water and once in 15% (v/v) methanol before use. All other reagents were of A.R. grade.

Preparation of mitochondria. Adult male albino rats were killed by dislocating the neck and the tissues rapidly homogenized in a Potter-Elvehjem homogenizer in a medium containing sucrose (0.25M) and EDTA (0.1mM), pH 6.8. A 10% (w/v) homogenate was centrifuged at 1000g for 6 min. at 0° to remove the nuclei and cell debris. To obtain the mitochondrial fraction the supernatant was then centrifuged at 10000g for 15 min. for the kidney and for 10 min. for the liver. The pellet was then washed once in the sucrose preparation medium and twice in the medium used for electrophoretic measurements before final suspension in that medium. All preparations were stored at 0° till required.

Determination of the electrophoretic mobility. All electrophoretic measurements were made in a medium of constant ionic strength (0.142). The standard medium used contained KCl (0.125M) and tris (0.02M) buffered to pH 7.4. For determination of the variation of mobility with pH the medium contained KCl (0.125M) and Michaelis (1931) buffer of ionic strength 0.02 and appropriate pH value. The mitochondria stored at 0° were diluted tenfold in the media at 25°, allowed to equilibrate for 3 min. and then introduced into the electrophoresis cell. Measurements of electrophoretic mobility were made in a flat rectangular chamber supported on the stage of a microscope and mounted between two electrodes. A known field strength was applied and mitochondria in focus at the stationary level were timed across a fixed distance. The apparatus and technique used was that of Gittens & James (1960).

Swelling of mitochondria. The rate of swelling of mitochondria was followed by measuring the decrease in extinction at 520mμ, in the medium used for electrophoresis, by using a Unicam SP.1400 spectrophotometer.

RESULTS

Stability of the electrokinetic surface

Effect of washing. After isolation the mitochondria were washed up to six times in the standard medium and their mobility was determined after each washing. Table 1 shows that there is no significant change in mobility after repeated washing of either rat-kidney or rat-liver mitochondria. From this the standard procedure of washing twice before measurement was adopted. Both rat-kidney and rat-liver mitochondria gave homogeneous distributions of mobility values with a mean of $-0.78(\text{s.e.m.} \pm 0.02) \mu/\text{sec.}/\text{v}/\text{cm.}$ (29 experiments) and $-1.06(\text{s.e.m.} \pm 0.01) \mu/\text{sec.}/\text{v}/\text{cm.}$ (21 experiments).

Mitochondrial swelling. Exposure of the mitochondria to 25° produced spontaneous swelling (Fig. 1) but no significant change in mobility (Table 2). Table 2 also shows the effect on the mobility after exposure of kidney mitochondria to thyroxine (Lehninger, 1956).

Table 1. *Effect of repeated washing on the mobility of mitochondria from rat kidney and liver*

Experimental details are given in the text.

No. of washings	Electrophoretic mobility ($\mu/\text{sec.}/\text{v}/\text{cm.}$)	
	Kidney mitochondria	Liver mitochondria
1	-0.69	-1.06
2	-0.80	-1.04
3	-0.81	-1.10
4	-0.84	-1.15
5	-0.86	-1.08
6	-0.79	-1.10

Fig. 1. Spontaneous swelling of mitochondria from rat kidney (●) and liver (○). A suspension of mitochondria in $0.125 \text{ M-KCl-}0.02 \text{ M-tris}$, $\text{pH } 7.4$, was exposed to 25° and the change in extinction at $520 \text{ m}\mu$ measured.

Effect of hydration and dehydration of the rat on the mitochondria of the kidney. Adult male rats deprived of food and water for 24 hr. were given water by stomach tube to 5% of their body weight. They were killed when the volume of urine excreted was about 60% of the administered water load, i.e. when the administered water had been reabsorbed from the digestive tract (Dicker, 1948). The kidneys were removed and the mitochondria prepared as described above. The mobility values were determined and compared with those for six rats that had been mildly dehydrated by depriving them of water for 48 hr. The results (Table 3) show that these extreme stresses thrown on the kidney do not affect the mitochondrial surface.

Change of mobility with pH

Measurements were made as described over the pH range 2.6–10.0 in medium of constant ionic strength (0.145). Fig. 2 shows the results obtained

Table 2. *Effect of swelling on the mobility of mitochondria from rat kidney and liver*

Experimental details are given in the text. The fall of extinction with time for spontaneous swelling of the mitochondria is that shown more fully in Fig. 1.

	Electrophoretic mobility ($\mu/\text{sec.}/\text{v}/\text{cm.}$)			
	Control 0–5 min. at 25°	Spontaneous swelling 55–60 min. at 25°	After 33 μM - thyroxine for 4 min.	Fall in $E_{520 \text{ m}\mu}$
Kidney mitochondria	0.80	0.83	—	0.45 → 0.33
Liver mitochondria	0.77	—	0.77	0.73 → 0.52
	1.06	1.00	—	0.60 → 0.48

Table 3. *Effect on the electrophoretic mobility of kidney mitochondria after hydration and dehydration of the rats*

Experimental details are given in the text.

Expt. no.	Electrophoretic mobility of mitochondria ($\mu/\text{sec.}/\text{v}/\text{cm.}$)		
	Control rats	Hydrated rats	Dehydrated rats
1	-0.78	-0.81	-0.78
2	-0.76	-0.78	-0.73
3	-0.72	-0.77	-0.80
4	-0.85	-0.84	-0.81
5	-0.80	-0.74	-0.80
6	-0.81	-0.82	-0.88
Average	-0.79	-0.79	-0.80

Fig. 2. Variation of the electrophoretic mobility with pH of mitochondria from rat kidney (Δ , $\blacktriangle$) and liver ($\circ$, $\bullet$) after exposure to 25° for 5 min. ($\circ$, Δ) and 60 min. ($\blacktriangle$, $\bullet$).

for liver and kidney mitochondria after exposure to 25° for 5 min. ($\circ$, Δ) and 60 min. ($\bullet$, $\blacktriangle$). As found for pH 7.4 (Table 2), exposure to 25° for 60 min. at various pH values does not affect the electrokinetic surface of kidney and liver mitochondria. The two curves obtained are very similar but differ in their isoelectric point; this is pH 3.9 for kidney and pH 4.4 for liver.

DISCUSSION

In any examination of particles by micro-electrophoresis it is essential to obtain a reproducible surface. It is important to see if material is adsorbed on to the surface from the medium or if a normal component of the surface is removed by washing (Plummer, James, Gooder & Maxted, 1962). Such changes, if they occurred, would result in alterations of the mobility value. The results obtained (Table 1) suggest that no loosely bound material is present on the surface of the mitochondria studied. Exposure to 25° or treatment with thyroxine does not alter the mobility value (Table 2), and thus no change in surface charge density is obtained. This suggests that swelling does not occur by stretching of the surface, since this would decrease the surface charge density and hence lower the mobility. The possible mode of swelling could be an unfolding of the surface. These present results agree with those of Thompson & McClees (1961), who found no change in surface charge density per unit area after exposing rat-liver mitochondria to a number of swelling reagents. The technique used in their experiments, however, was that of free-boundary electrophoresis at 0° in hypo-osmotic media. In addition, the results obtained by Pauly & Packer (1960) support the above conclusions. These authors found no change in resistance or capacitance of the mitochondrial membrane with swelling.

The mitochondrial membrane provides a useful model for the study of the action of neurohypophyseal hormones, as they cause swelling of mitochondria *in vitro* at concentrations in approximately the physiological range (Greenbaum & Dicker, 1963). The results obtained on the kidney mitochondria from rats in water diuresis or during antidiuresis indicate that the absence or presence of circulating neurohypophyseal hormones does not permanently affect the mitochondrial surface as detected by micro-electrophoresis. At the ionic strength used (0.142) this means that charged groups within 10 Å of the surface are unchanged (Bangham, Pethica & Seaman, 1958).

Homogeneous distribution of mobility values were obtained from rat-kidney and rat-liver mitochondria, and thus mitochondria prepared from a single tissue would appear to have a similar surface. The different swelling response of mitochondria from the kidney cortex and medulla to posterior-pituitary hormones (Greenbaum & Dicker, 1963) has to be explained in terms other than large overall differences in the ionogenic groups. It has been suggested that the disulphide hormones cause swelling of mitochondria by reacting with certain thiol groups of the membrane (Lehninger & Neubert, 1961; Fong, Silver, Christman & Schwartz, 1960), and Riley & Lehninger (1964) have shown that during swelling of rat-liver mitochondria by oxidized glutathione a fall in the number of membrane thiol groups occurs. The difference in sensitivity of mitochondria from the kidney cortex and medulla might therefore be due to a different content or pattern of thiol groups.

Rat-kidney and rat-liver mitochondria show a similar variation of mobility with pH, though their isoelectric points are pH 3.9 and pH 4.4 respectively. The presence of an isoelectric point indicates that each surface contains both acidic and basic groups. This agrees with the findings of Douglas *et al.* (1963), who obtained an isoelectric point of about pH 4.2 for rat-liver, bean and cauliflower mitochondria. As these authors have shown that mitochondria prepared from such diverse sources all contain similar surface charged groups, so in this present study similar ionogenic groups have been found on rat-kidney and rat-liver mitochondria. Thus it may be that mitochondria in general have a common type of ionogenic surface. The reason for the different response to various swelling reagents may therefore be due to other properties of the membrane or to varying amounts of endogenous substrate in the different mitochondrial preparations.

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