Table I. Effect of Sulpiride on oxygen uptake by brain slices in vitro

Drug concentration	Potassium concentration (mM)	Calcium concentration (mM)	Oxygen uptake (μl/100 mg wet tissue/h)
Control	5	2.5	64.56 ± 3.61 (10)
	100	2.5	78.21 ± 2.76 (12) [c]
	5	0	69.50 ± 4.68 (9)
1×10^{-2} M	5	2.5	53.62 ± 4.41 (10) [a]
	100	2.5	47.10 ± 3.19 (12) [b]
	5	0	52.49 ± 3.13 (9) [c]
1×10^{-3} M	5	2.5	69.40 ± 7.17 (11)
	100	2.5	69.95 ± 3.11 (12)
	5	0	70.08 ± 4.68 (9)
1×10^{-4} M	5	2.5	61.40 ± 6.53 (8)
	100	2.5	78.65 ± 3.76 (10)
	5	0	68.09 ± 6.83 (9)

[a] $P < 0.02$; [b] $P < 0.001$; [c] $P < 0.01$. The figures are means ± SEM. In brackets the number of slices.

Table II. Effect of Sulpiride on oxygen utilization by brain homogenates

Drug concentration	No. of experiments	Oxygen uptake (μl/100 mg wet tissue/h)
Control	12	54.77 ± 3.14
1×10^{-2} M	8	43.91 ± 1.99 [a]
1×10^{-3} M	7	52.77 ± 4.49
1×10^{-4} M	8	53.61 ± 4.81

[a] $P < 0.025$. The figures are means ± SEM.

Results. The data on the oxygen uptake by brain slices are shown in Table I and shows that, under control conditions, in Krebs-Ringer phosphate medium with high concentration of potassium (100 mM), the oxygen uptake is increased up to 21.1%. The absence of calcium in the medium does not change significantly the oxygen consumption.

Sulpiride, at high concentrations (10^{-2}), inhibits significantly the oxygen uptake at 60 min of incubation; this inhibition appears greater when the medium contained high potassium concentrations.

The data on the oxygen uptake by brain homogenates are shown in Table II; the results show that Sulpiride at highest concentration (10^{-2}) significantly inhibits the oxygen utilization. Lower doses of sulpiride (10^{-3} and 10^{-4}) do not have any significant inhibitory action on the oxygen uptake, either by brain slices or by brain homogenates.

Discussion. The excess of potassium in the incubation medium increases the oxygen uptake in rat's brain slices. These data agree with those of ASHFORD and DIXON[11] and DICKENS and GREVILLE[12]. According to our data, the absence of calcium in the medium, using air as gas phase, did not enhance the oxygen utilization. These results are in disagreement with BUCHEL's data[2], but this author used O_2 as gas phase.

Sulpiride appears to have a depressor effect on the oxygen uptake in vitro only at very much higher concentrations than those which might be considered pharmacologically active. For that reason, we cannot suppose that the inhibition of oxygen uptake induced by Sulpiride will be the mechanism of its action on the central nervous system. This inhibiting effect appears more evident when the oxygen uptake was previously stimulated by higher potassium concentrations in the medium, in such manner as occurs with several central nervous system depressors in the same experimental conditions[5,6]. However, the presence or the absence of calcium in the medium did not influence the depressor effect of Sulpiride on the oxygen uptake in vitro.

Resumen. El Sulpiride in vitro a la concentración de 1×10^{-2} M disminuye el consumo de Oxígeno en cortes de cerebro de rata. El exceso de potasio o ausencia de calcio en el medio de incubación, no modifican este efecto. En homogeneizado de cerebro total, unicamente a la concentración de 1×10^{-2} M deprime el consumo de oxígeno. A las concentraciones 1×10^{-3} y 1×10^{-4} M no modifica el consumo de oxígeno en cortes ni homogeneizados de cerebro.

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Ultrastructure of Renal Collecting Tubules Following Ingestion of a Bipyridinium Herbicide (Morfamquat)

Morfamquat dichloride (MFQ), 1, 1′-*bis*(3,5-dimethylmorpholinocarbamylmethyl) 4,4′ bipyridylium dichloride dihydrate, is a herbicide for farm and garden[1]. Similar to other bipyridinium compounds it reacts with the atmospheric O_2 to form labile hydroperoxides, which in turn give off activated oxygen.

High toxic doses of MFQ produce degenerative changes and necrosis of the proximal convoluted tubules in the kidneys of rats and dogs, whereas a low dietary concentration (0.015%) has been reported by FERGUSON et al.[2] to cause a marked increase in the number of mitochondria in the epithelial cells of the collecting ducts of Alderley Park strain rats.

A phenomenal increase in mitochondria is the hallmark of the oncocyte, a peculiar epithelial cell type seen in various human organs[3]. Since the factors leading to the development of oncocytes are not known and animal models for them have not yet been found, the experimental production of oncocyte-like cells appeared to possibly offer insight into the ultrastructural changes preceding or accompanying their development. The present communication reports the results of an attempt to induce mitochondrial proliferation in collecting duct cells by feeding low dietary concentrations of MFQ.

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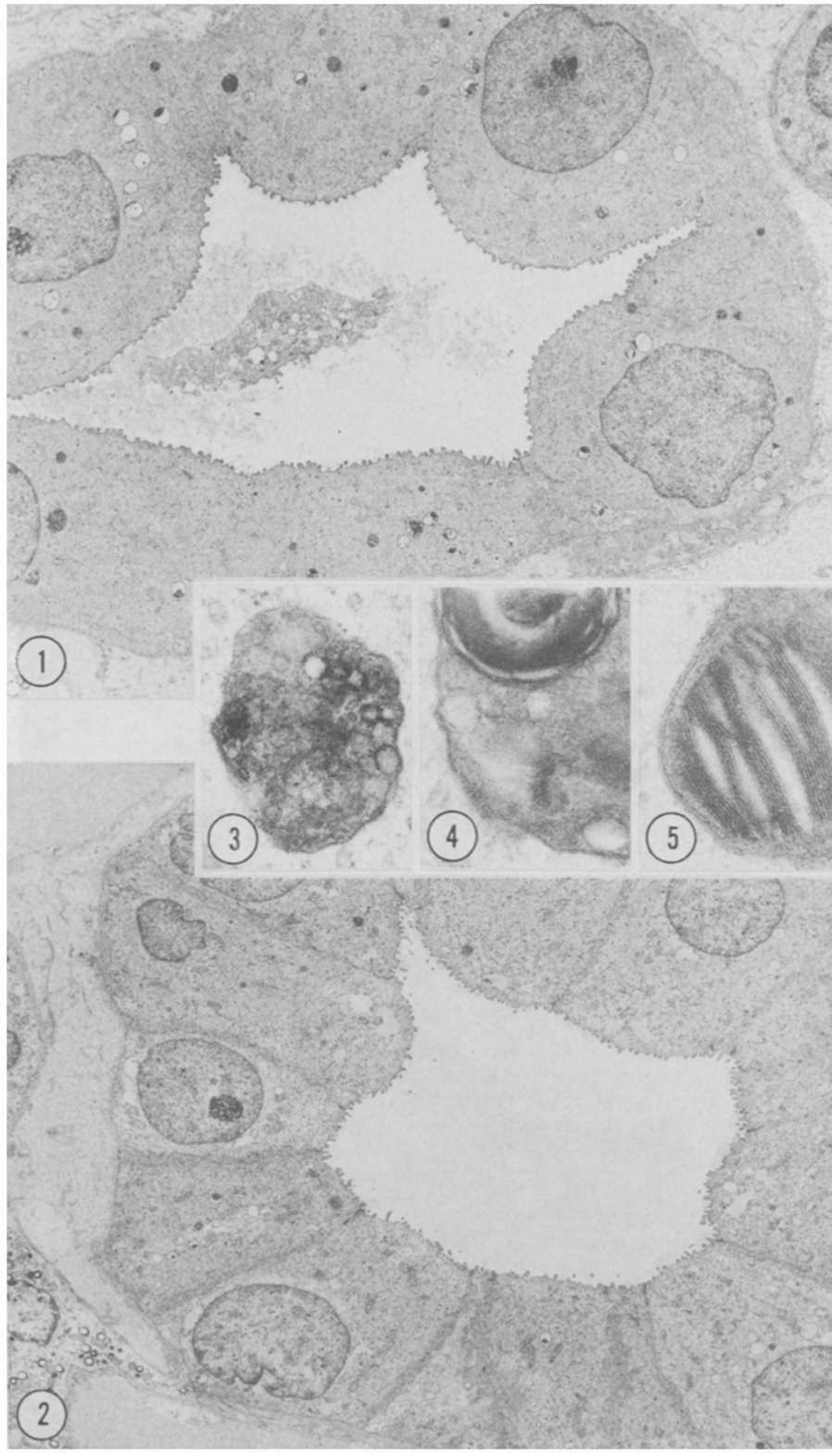

Fig. 1. Cross section of a collecting duct from the kidney of a rat fed Morfamquat diet for 24 weeks. Many electron dense bodies (some partly extracted) are found in the cytoplasm of cells lining the duct. A considerable quantity of cellular debris is present in the lumen. × 4,100.

Fig. 2. Collecting duct from a rat on the control diet. A few electron dense bodies can be observed in the cytoplasm. No debris is seen in the lumen. × 3,700.

Figs. 3, 4 and 5. Detailed views of dense bodies within the cytoplasm of duct cells from experimental animals. Vesicular inclusions (Figures 3 and 4) and whorls of packed 'myelin' membranes (Figures 4 and 5), exhibiting electron dense and lucent bands 25 A wide, are found within some of the bodies. Fig. 3. × 68,000; Fig. 4. × 80,000; Fig. 5. × 112,000.

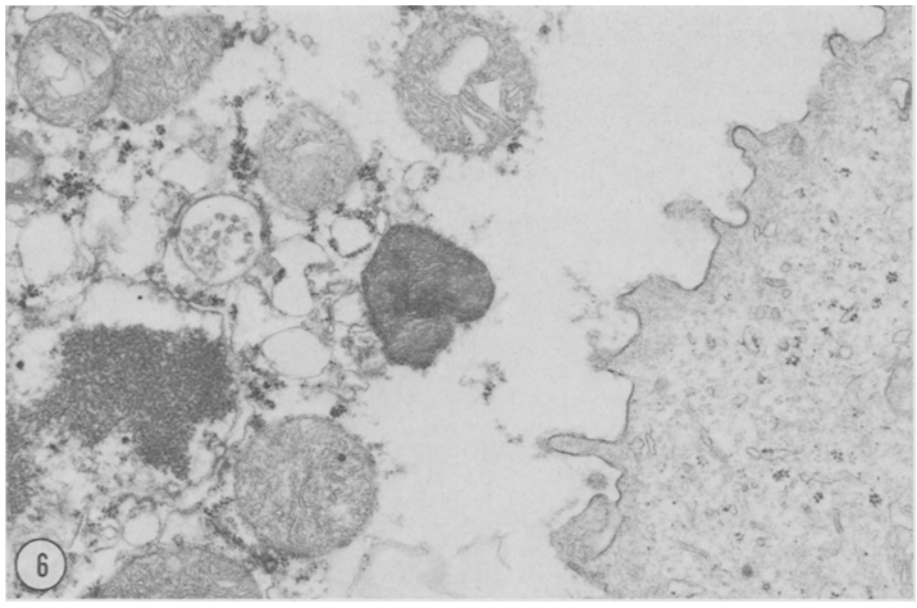

Fig. 6. Cellular debris, which has been proximally discharged into the nephron of an experimental rat, is observed adjacent to the luminal surface of a collecting duct cell. Remnants of a nuclear envelope and adhering chromatin is seen as well as mitochondria and a multivesicular body. × 29,000.

Eighteen 2-month-old male Sprague-Dawley rats were maintained on a special diet consisting of 5.75 kg rat chow meal, 1.35 kg ground malt and 375 cm³ corn oil mixed with 7,500 cm³ of boiling water containing 225 g agar. The above ingredients were blended into a uniform mash by means of a heavy duty mechanical mixer. To make the experimental diet, 1.384 g morfamquat dichloride salt (1.25 g free cation) was dissolved in water and was thoroughly mixed into the mash after it had cooled to 45 °C (final concentration of herbicide = 0.015% W/W). Control and experimental diet was pressed into cubes which hardened upon air drying at room temperature. The animals had free access to the diet and to water.

Three rats maintained on the experimental diet and 3 rats on the control diet were sacrificed by decapitation at 6, 12 and 24 weeks after initiation of the experiments. Samples from parotid gland, thyroid, liver and renal papilla were fixed for electron microscopy by immersion in phosphate buffered glutaraldehyde for $2^1/_2$ h. Following a buffered rinse, the tissues were fixed for 4 h in cold phosphate buffered 2% osmic acid and were dehydrated in an ascending ethanol series. The specimens were embedded in Epon 812, sectioned on an LKB ultramicrotome, and after sequential staining with uranyl acetate and lead citrate were examined with a Philips 300 electron microscope.

No significant ultrastructural changes were noted in the above mentioned tissues, except the kidney where our study was limited to the collecting ducts. Changes were most apparent in the 24 week experimental animals and were constant. When the epithelial cells, lining the ducts of the experimental animals (Figure 1) were compared with those of control animals (Figure 2) an overall increase in lysosomal bodies was noted. These lysosomes, which appeared similar to the electron dense bodies reported by MILLER and PALADE[4], ranged from approximately 0.2–1.2 μm in diameter. They were bounded by single unit membranes and often contained small vesicular inclusions (Figure 3) or whorls of packed membranes (Figures 4 and 5). Based on morphological criteria, the structures should be classified as multivesicular or residual bodies[5]. The lysosomes which we have found in the collecting duct cells closely resemble the cytosomes reported by FOWLER and BROOKS[6] in proximal tubules of mouse kidneys following the feeding of Paraquat, another bipyridinium herbicide.

In addition we observed considerable quantities of cellular debris within the lumina of the collecting ducts. Although erythrocytes were occasionally noted, cell organelles including pyknotic nuclei, mitochondria and lysosomes appeared more frequently (Figure 6). The numbers and size of mitochondria within the duct cells were not significantly different from that of the controls; nor was there any evidence of intramitochondrial changes.

Although the diet used in our experiment was similar to the one used by FERGUSON et al.[2] and the duration of our experiment was greater, we could not observe the mitochondrial proliferation reported by them. The reasons for this discrepancy are not clear; perhaps the strain difference may be significant. Since we observed no cellular disruption at the level of the collecting ducts in the renal papillae, we have concluded that the discharge of debris has occurred more proximally in the nephron. Indeed, extrusion of cell structures into the lumen by proximal tubule cells has been reported[6] under similar circumstances.

Zusammenfassung. Ratten, denen eine Diät mit niedrigem Gehalt von Morfamquat gefüttert wurde, zeigten nach 6–24 Wochen eine erhöhte Anzahl von Lysosomen in den Epithelzellen der Nierensammelröhren. Im Lumen waren Zelltrümmer zu sehen, die auf eine Zellnekrose im proximalen Nephron schliessen liessen. Die Mitochondrien wiesen weder quantitative noch qualitative Veränderungen auf.

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