packed membrane system. Giant mitochondria were observed, however, in rare instances. As shown in Figure 3, giant mitochondria can appear within an otherwise normal cell containing normal mitochondria. Such giant mitochondria may have diameters 2 or 3 times in excess of normal mitochondria, and lack the dense mitochondrial matrix characteristic of this cell. Although it appears that the number of crystae are not increased and that the internal membrane system remains intact in giant mitochondria, a degree of degenerative vacuolization is apparent.

Discussion. Crystalline structures are not commonly observed in parietal cells of any species. The only previous report of cytoplasmic crystalloids in parietal cells was that of WINBORN and BOCKMAN[4]. These investigators observed intramitochondrial crystals in hamster parietal cells, inclusions which appeared to be degenerative by-products of the mitochondria, and which were contained within a sectional area of mitochondrial size. In addition, HELANDER[11] recently reported the appearance of crystalline structures in the secretory product of parietal cells of weanling rats, a material which was present in the intracellular canaliculi and lumen of the fundic glands. Although the intracellular crystalline material observed in the case of the ferret may be the same as that previously reported[4], we have no evidence of it arising from mitochondria. Intramitochondrial crystalloids have also been reported in other cell types[12–14]. Crystal containing cells of the mouse large and small intestine have been reported[15], but such cell types are presumably of migratory origin[16]. The crystalline material observed in the ferret parietal cells is probably distinct from the oxyphilic substance noted many years ago by ZIMMERMANN[17], and considered as a precursor of hydrochloric acid. Indeed, it is currently held that hydrochloric acid is not stored, and is formed within the membrane system of the intracellular canaliculus.

Giant mitochondria have not been reported previously in parietal cells. The normal integrity of the parietal cell illustrated in Figure 3, as well as the conventional appearance of most of the mitochondria, suggests a pleomorphism of mitochondria in this cell. Such enlargement of mitochondria, observed here in an untreated ferret, has not previously been noted in our laboratory in ferrets treated by a variety of ulcerogenic drugs[18].

Résumé. On décrit deux rares variations structurelles: cristalloïdes cytoplasmiques intracellulaires et mitochondries géantes, observées dans les cellules marginals de la muqueuse gastrique du furet. Les cristalloïdes cytoplasmiques sont probablement en rapport avec la dégénération mitochondrielle, et les mitochondries géantes sont un caractère aberrant.

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Effect of Acriflavin on the Mitochondria of the Rat

Studies of the last few years have demonstrated that the antitrypanocidal drug acriflavin provokes alterations of the mitochondria in yeast[1,2] and of the kinetoplast and mitochondria in members of the family Trypanosomatidae[3–6]. To our knowledge no studies have yet been performed on the effect of this drug on the fine structure of mammalian cells. This fact induced us to find out if acriflavin could provoke in a higher species mitochondrial modifications similar to those reported in unicellular organisms.

Thirty male rats of the Wistar strain were injected i.p. with 10 mg/kg body wt. of neutral acriflavine (British Drug Houses Ltd.). The dose was chosen after a preliminary test of toxicity. Groups of 5 animals each were killed at 1, 2, 4, 6, 12 and 24 h after the injection of acriflavin. As controls 2 non-injected animals were used for each group. Samples of liver, heart and kidney were fixed in 1% osmium tetroxide in phosphate buffer and processed for the electron-microscopic study. Thin sections were stained with uranium acetate-lead citrate, with lead citrate alone or with potassium permanganate.

In the animals belonging to the 4 and 6 h groups, the fine structure of the parenchymal cells of the liver, kidney and myocardium did not show alterations and appeared normal according to currently accepted morphologic criteria. The only modification found consisted in the appearance of clear areas within the mitochondrial matrix. These electron-lucid zones were approximately circular in shape and measured 210 nm in mean diameter. Within the clear areas thick fibers from which emerged thin fibrils were frequently seen. The fibers stained heavily with uranium. In alternate sections stained with lead alone, or with potassium permanganate, the fibers were hardly apparent. Generally only one electron-lucid zone was seen per mitochondrion, but occasionally mitochondria with 2 clear areas were observed. The general morphology and the affinity for the uranium stain of the intramitochondrial fibers was similar to those reported in other plant and

animal species and considered to be mitochondrial DNA[7,8]. The number of DNA-containing mitochondria was variable for each organ. The myocardium and the liver showed a higher incidence than the kidney. In the heart muscle cells up to 5% of the mitochondrial profiles presented DNA fibers, and in the hepatocytes as many as 10% of the mitochondria showed DNA. In the remaining groups and in the control animals, the DNA-containing mitochondria were very scarce and represented less than 1% of the whole mitochondrial population.

Apparently the administration of acriflavin, in the dose employed in the present work, provokes a marked increase in the number of mitochondria containing DNA fibers in different organs of the rat. Although with the present evidence the explanation of this fact is not clear, some alternative explanations could be pointed out. It is rather difficult to establish whether the appearance of intramitochondrial filaments of DNA corresponds to a spiralization and condensation of the DNA already existent in the mitochondria, which becomes in this way visible at the ultrastructural level, or if acriflavin stimulates the synthesis of new mitochondrial DNA, rendering it apparent. From data reported in unicellular organisms, it appears that acriflavin prevents the replication of the kinetoplast[5,6] and mitochondrial[6] DNA, probably by its ability to combine with DNA molecules with a high adenine-thymine base pair composition[9]. If this is also the case for the rat cells, acriflavin would prevent the synthesis of new mitochondrial DNA, and its visualization would be the result of a condensation of pre-existent DNA, unable to replicate owing to the drug action. Accordingly, the increase in the number of mitochondria with DNA fibers after acriflavin treatment would be the result of an inhibition of the DNA synthesis.

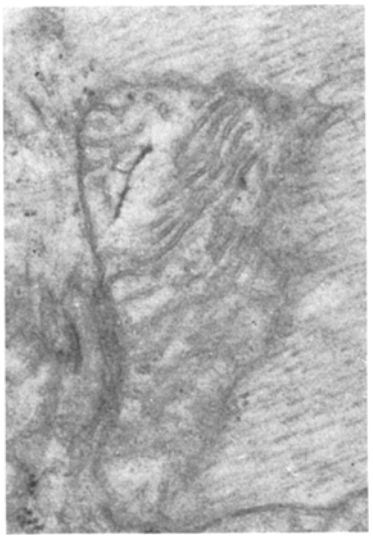

Fig. 1. Heart mitochondrion. A long and thick fiber heavily stained with uranium can be seen. × 43,000.

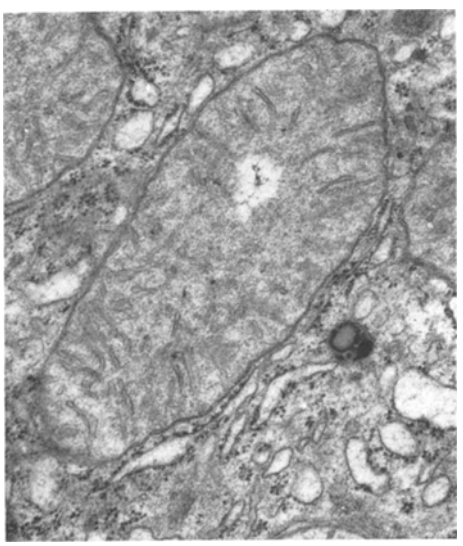

Fig. 2. Liver mitochondrion. An electron-lucid zone containing electron-dense filaments may be observed. × 36,000.

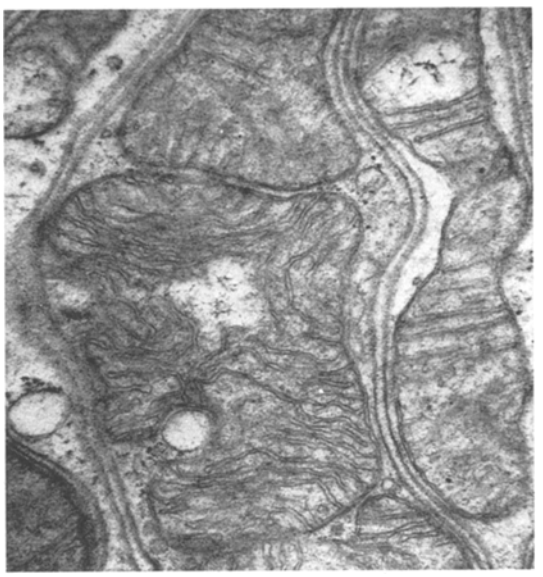

Fig. 3. Mitochondria of the distal tube of the rat kidney. In both of them clear zones containing fibers and filaments are visible. × 44,000.

This fact induces us to consider the possibility of using acriflavin as an 'antimitotic' drug for mitochondrial DNA, permitting the determination of the 'mitotic time' and 'mitotic index' of the mitochondrial population. Studies are at present in progress to test this possibility.

Resumen. La administración de acriflavina provoca la aparición de un elevado número de mitocondrias con filamentos de ADN en el hígado, riñón y miocardio de la rata. Sobre la base de este hecho se sugiere que la acriflavina inhibe la reproducción del ADN mitocondrial, haciéndolo visible a nivel ultraestructural.

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