Journal of Veterinary and Animal Sciences. 25 (1994) 25-32

MITOCHONDRIAL CHANGES IN THE KIDNEY AND LIVER OF MICE INDUCED BY OCHRATOXIN A - AN ELECTRONMICROSCOPIC STUDY

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Ochratoxins and toxic compounds commonly produced by the fungi, *Penicillium verrucosum* Dieckh and *Aspergillus ochraceus* Wilhelm. The latter is now referred to as *Aspergillus alutaceus* Berkley et curtis. Of these, ochratoxin A (OA) is the most toxic type. The target organ of OA is the kidney, although other organ systems can also be severely involved (Marquardt and Frohlich, 1992). This mycotoxin has also teratogenic, carcinogenic and immunosuppressive effects in many species of animals (Arora, 1983, Kuiper-Goodman and Scott, 1989 and Huff, 1991). The cause of cell damage by OA has been attributed to different mechanisms. It has been suggested that OA interferes with phenylalanine metabolism, causes lipid peroxidation and affects mitochondrial function (Meisner and Chan, 1974; Meisner and Meisner, 1988; Rahimtula *et al.*, 1988 and Aleo *et al.*, 1991). Since mitochondria are directly involved in many metabolic functions of cells, ultrastructural alterations in this organelle in renal and hepatic cells of mice after experimental oral feeding with OA, were evaluated.

Materials and Methods

OA was prepared from *Aspergillus ochraceus* (from the culture collections of Federal Meat Research Institute, Kulmbach, Germany) by the standard methods. Ten to twelve week old NMRI strain of mice were used for the study. Ten mice were given feed containing 20 ppm of the toxin. A second group of 10 mice was kept as control and fed with standard diet without incorporation of the toxin. Feed and water were given *ad lib.* The weight of the animals was recorded just before the start of experimental feeding and at sacrifice on the 22nd day. The quantity of the feed consumed was also assessed. Tissues, after sacrifice of the animals were collected and fixed in 3% glutaraldehyde in cacodylate buffer. The tissue samples from liver and kidney were processed for electronmicroscopy and thin sections cut in an LKB microtome. They were examined and photographed in a Hitachi 600-A electronmicroscope after staining with uranyl acetate and lead nitrate.

Results and Discussion

The weight of OA fed animals showed a significant reduction at the time of sacrifice on the 22nd day from a mean weight of 40.13 g to 26.35 g. The corresponding figures for the control animals were 36.86 g and 35.22 g respectively. During the period of the experiment each mouse consumed an average of 0.052 mg of OA daily which was below the acute toxic level. The acute oral toxicity of OA when expressed as LD_{50} values is 46-58 mg/kg body weight (Marquardt and Frohlich, 1992).

The ultrastructural changes seen in the cells of the proximal and distal convoluted tubules were more severe than in the other parts of the nephron. Mitochondria of the proximal convoluted tubules were pleomorphic showing diverse form and structure (Fig.1). They were either elongated with longitudinal cristae or forked or dumbell shaped or assumed a curved shape (Fig.2). Many of them had a circular profile and they had a concentric arrangement. In

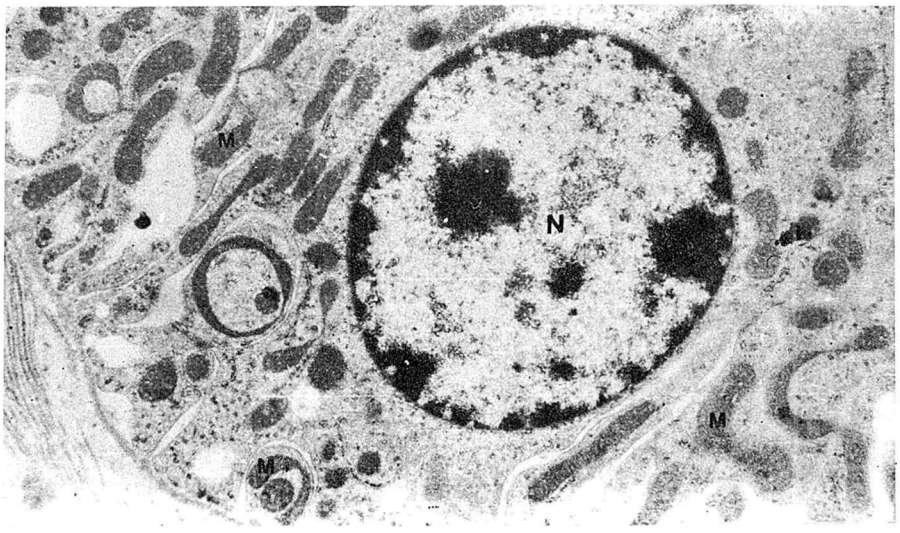

Fig.1 Kidney - proximal convoluted tabule epithelial cell showing pleomorphic mitochondria (M). N - Nucleus x 12,000

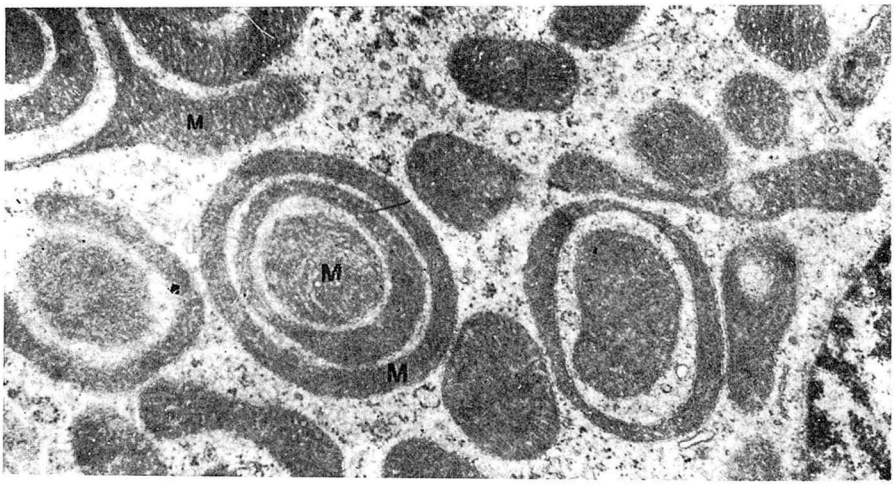

Fig.2 Kidney - proximal convoluted tubule epithelium. Mitochondria (M) showing morphological variations. Some of them are arranged concentrically. There is partial or complete loss of cristae x 30,000

many mitochondria the outer membrane appeared intact. Occasionally focal separation of inner and outer mitochondrial membranes was observed. Cristae showed disruption. Partial or complete lysis of cristae or distorted cristae were also noticed. Mitochondrial matrix in general, was intensely electrondense with comparative loss of matrix granules. Such aberrations of mitochondria were also seen in the epithelium of the distal convoluted tubules, but they were not as intense as seen in the proximal tubules. The orientation of the mitochondria along the infoldings of the basal plasmalemma was maintained eventhough many of these appeared swollen (Fig. 3). Matrical granules were occasionally seen. The changes in the glomerulus were of moderate intensity with only occasional fusion of the foot processes of podocytes. Mitochondrial changes in the endothelium, podocytes and mesangial cells were mild with swelling and occasional loss of cristae. The tubular epithelial cells showed a moderate increase in the number of peroxisomes.

In the liver, one characteristic observation seen was the preponderance of lipid granules in the cytoplasm (Fig. 4). The mitochondria were uniformly swollen, eventhough in many, the outer mitochondrial chamber could be clearly visualized. The outer membrane appeared intact. Many mitochondria had lost the integrity and structure of cristae and the matrix appeared uniformly electron-dense with occasional remnants of cristae. There was an increase in the number of peroxisomes and smooth endoplasmic reticulum (SER) (Fig. 5 and 6). Increase of peroxisomes has been reported in a variety of toxic conditions (Ghadially, 1982). It has been suggested that peroxisomes might have evolved as a protection since the catalase content can convert the deleterious hydrogen peroxide. The proliferation of SER could be a response to the toxic metabolites present in the cell since the SER of the liver contains enzymes that catalyze the detoxification of drugs and harmful metabolic products. The other ultra-structural changes in the nucleus, nucleolus and rough endoplasmic reticulum were similar, though lesser in intensity to those described earlier in the liver and kidneys of broiler chicks (Dwivedi and Burns, 1984), chicken embryos (Lalithakunjamma, 1987) and goats (Maryamma and Nair, 1990 a, b).

The ultrastructural alterations definitely point to the effect of OA on mitochondria. It was reported that OA inhibited the respiration of whole mitochondria by acting as a competitive inhibitor of carrier proteins located in the inner mitochondrial membrane (Meisner and Chan, 1974). The severe changes in the epithelial cells of the convoluted tubules may be due to the fact that apart from enterohepatic recycling, OA is also resorbed in an acute manner via the organic anion transport system in the proximal and distal convoluted tubules of the kidney (Marquardt and Frohlich, 1992). It has also been reported that the primary effect of OA is on the enzymes involved in phenylalanine metabolism including pheylalanine transferase and hydroxylase. It has been shown that OA stimulated lipid perodixation and the iron complex of OA produced an extremely toxic hydroxyl radical in the presence of NADPH - cytochrome C-P-450 reductase system. The lipid peroxidation may cause structural changes in the membrane system of cell, including that of mitochondria, resulting in an influx of cellular calcium leading to metabolic alterations (Orrenius and Bellome, 1986). There is evidence to show that OA caused a depletion of intramitochondrial ATP and inhibited mitochondrial phosphate transport (Suzuki *et al.*, 1975). This may result in condensation of matrix protein. The mitochondrial swelling may be due to ionic shifts that occur in the mitochondrial inner compartment.

The inner mitochondrial membrane is the site of the electron transport chain which contains acceptors to which electrons from the citric acid

cycle and β oxidation of fatty acids are transferred. Similarly the mitochondrial matrix is the site of reactions of the citric acid cycle. The size and shape of the mitochondria depends greatly on their physical and metabolic environment and large mitochondria are produced under the influence of nutritional deficiencies and toxic factors. It has been shown that abnormal mitroschondria exhibited abnormal functions with uncoupling of oxidative phosphorylation and a specific decrease in enzymatic activity of the respiratory chain complex (Lamperth *et al.*, 1991). The morphological alterations seen in the mitochondria in this study clearly indicate that OA has a direct toxic action on the mitrochondria which would result in aberrations of biochemical functions. Biochemical studies would be needed to assess at what stage of the respiratory chain system, the uncoupling takes place. It is also possible that OA inhibited the DNA synthesis. A measurement of the mitochondrial DNA would indicate whether there has been reduced DNA synthesis.

Neoplasms in the kidney is one of the effects of OA (Benedele *et al.*, 1985) and probably if the experimental period was prolonged sufficiently long, carcinogenic effects would have manifested.

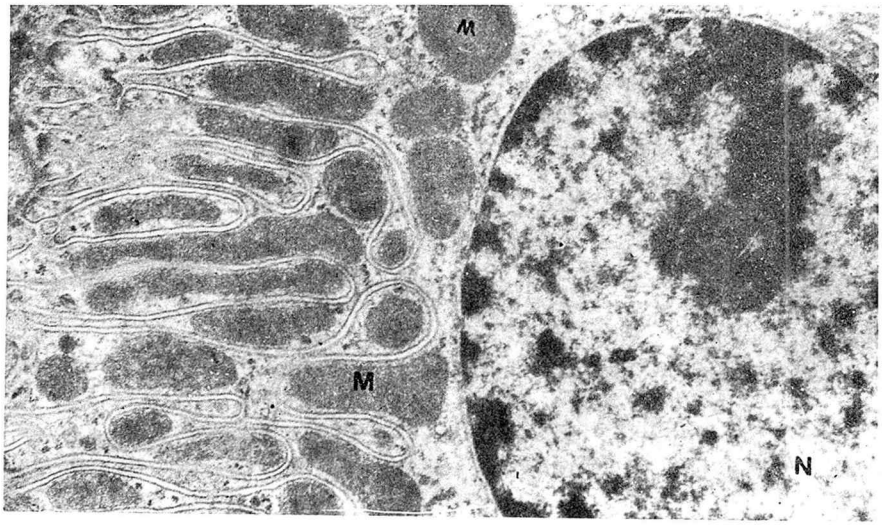

Fig.3 Kidney - proximal portion of an epithelial cell of the distal convoluted tubule - swollen mitochondria (M) with an electron dense matrix and loss of cristae x 25,000

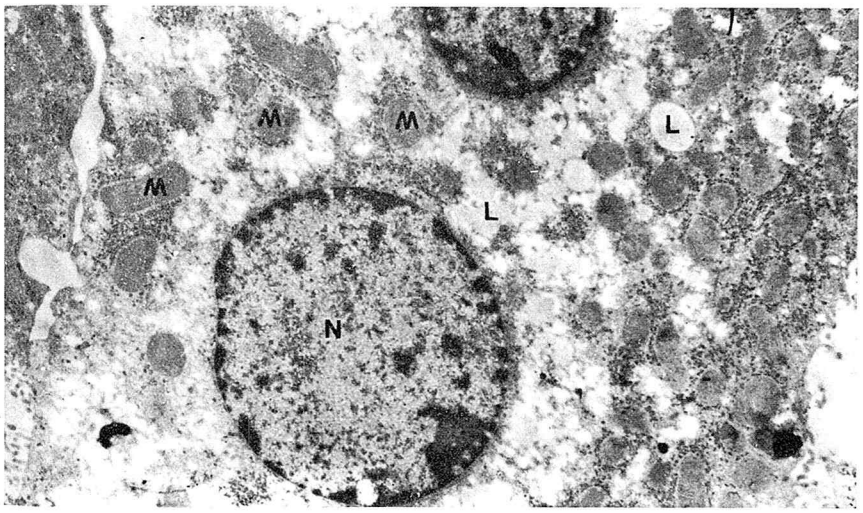

Fig.4 Liver - Hepatic cell showing numerous lipid droplets (L) and swollen mitochondria (M). N - Nucleus x 12,000

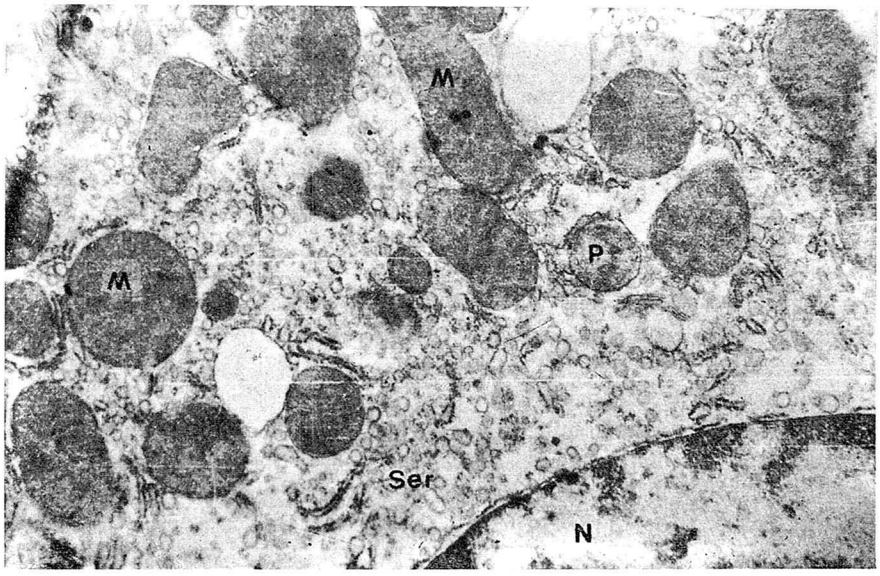

Fig.5 Liver - Hepatic cell showing swollen mitrochondria (M) and peroxisomes (P). There is proliferation of smooth endoplasmic reticulum (Ser.). N - Nucleus x 18,000

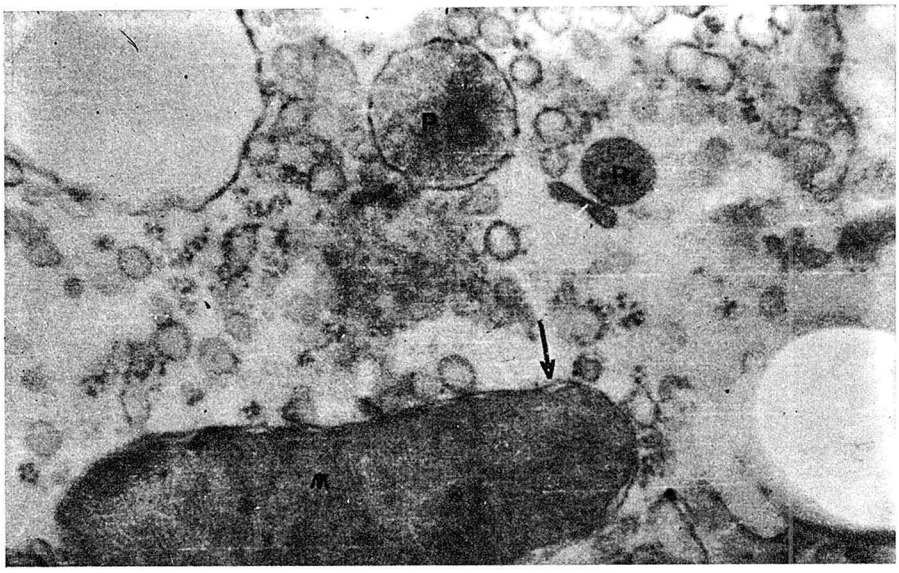

Fig.5 Liver - Hepatic cell showing swollen mitrochondria (M) and peroxisomes (P). There is proliferation of smooth endoplasmic reticulum (Ser.). N - Nucleus x 18,000

Summary

Ultrastructural changes of renal and hepatic mitochondria in mice induced by OA were studied. Mitrochondria exhibited diverse morphological aberrations. The outer mitochondrial membranes, cristae and matrix were affected. The changes were more prominent in the proximal and distal convoluted tubules of kidney. In the liver, besides the mitochondrial changes, there was proliferation of SER. An increase in the peroxisomes was observed in the kidney and liver. The morphological aberrations seen in the mitochondria indicate that OA has a direct toxic action on the mitochondria causing aberrations of mitochondrial function resulting in metabolic derangement in the cell.

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