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## KIDNEY LESIONS FROM MYCOTOXINS AS OBSERVED BY ELECTRON MICROSCOPE

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### Résumé

OBSERVATIONS AU MICROSCOPE ELECTRONIQUE DES LESIONS RENALES PROVOQUEES PAR LES MYCOTOXINES. — Les modifications histopathologiques du rein provoquées par les mycotoxines présentes dans le foin sont étudiées au microscope électronique. Des changements sont observés dans les mitochondries, les membranes, la granulation plasmatique, la sécrétion du glycogène, la vésicularisation et la vacuolisation.

A part of the hay crop becomes mouldy during harvest time as a consequence of climatic conditions. Feeding mouldy hay pellets or drinking its extract (Shadmi *et al.*, 1974) resulted in a growth retardation of 30 % ( $P = 0.05$ ) in 60 day juvenile rats and 36 % ( $P = 0.05$ ) in 80 day old kids.

Histological sections revealed 17 cases of lesion in the kidneys of 24 animals. No such changes were found in the animals fed normal hay. A histological survey of the kidneys of 9 animals (4 mice, 4 rats, 1 kid) injected with mouldy hay extracts showed 78 % pathological findings compared with 11 % in the animals injected with normal hay extracts.

### Materials and Methods

#### Animals

Albino mice were raised to the age of 20

days before the experiment was begun. After weaning they were fed on standard « Ambar » pellets for mice. Before the day of experiment, mice of average weight of 10 g were chosen.

*Mouldy hay, the ether soluble fraction of methanol extract, and treatment of the mice.*

Freshly baled vetch hay was artificially wetted to contain 40 % water. The hay was allowed to ferment naturally and bales fermented at not less than 45 °C were chosen for further study.

The isolation of moulds from normal and mouldy hay was carried out on Czapek medium (Plant Pathologist's Pocketbook, 1968) at three incubation temperatures (25, 35, and 45 °C). The character of the population varied with the incubation temperature. For example *Aspergilli* and *Actinomyces* increased greatly at 45 °C. About 16 species of the genera *Aspergillum*, *Penicillium*, *Fusarium* and

others were identified<sup>1</sup>, of which seven are known to be toxic (Bamburg *et al.*, 1969). Of these seven, five were isolated only from mouldy hay. The three toxic species of *Aspergillum*, *Asp. flavus*, *Asp. ochraceus* and *Asp. fumigatus* were further investigated. The thermophilic character of the last named fungal species suggests that it may be dominant in hay that undergoes hot fermentation. Methods of fermentation, methanol extraction and separation of the ether-soluble fraction, were performed as previously described (Shadmi *et al.*, 1974).

The ferment was analysed according to the methods (Packter, 1965; Pons *et al.*, 1966; Scott and Hand, 1967) and found to contain 2 ppm aflatoxin, 2 ppm ochratoxin and 120 ppm fumigatin-fumigatol respectively on the basis of the dry weight of hay powder.

The dried ether-soluble fraction of methanolic extract was dissolved in propylen-glycol (600 mg in 10 ml). One intravenous injection of the solution, which contained 6 mg dry matter (equivalent to 400 g of the hay powder per 1 kg live weight) was given to each of 24 albino mice. As a control 6 albino mice were injected with increasing doses of normal and mouldy hay extract (ether soluble fraction) to equivalent of 1500 g of hay powder per 1 kg live body weight.

The injections were carried out with the aid of tuberculin small injectors (1 ml) and Luer BD No 26 needles. The solution was not autoclaved but filtered with Millipore Swinnex 13 Filter Unit (Bedford, Mass.). The injections were given intravenously in the tails, which were rubbed immediately before with xylene.

The treated animals and the controls were sacrificed 24 h after the injection and their kidneys were excised for parallel examination by light — and electron microscopy.

#### *Preparation and staining for electron microscopy*

Specimens for light microscopy were fixed in 10 % formalin and dehydrated with alcohol and paraffin. Sections were stained with haematoxylin-eosin.

The collected tissue for electron microscopy was sectioned into small cubes approximately

1mm in length. Fixation was performed as described (Dalton, 1955). The tissue was treated with a 5 % glutaraldehyde solution, dissolved in 0.2 M Na-cacodylate buffer pH 7.4. After this treatment the samples were soaked in chromosmium solution for 24 hours. Dehydration was performed by the method with ethyl alcohol and propylene oxide (Culling, 1963). Embedding was performed in plastic envelopes in Epon 812 with DPM hardener. The blocks were scraped with pyramitome and thin cuts were obtained with the Alvir ultramicrotome. Slices were then fixed on metallic grids (reticulated 400 mesh) and were stained with uranyl-acetate and lead citrate.

Observation of the prepared tissue was done as directed for electron microscopy by catalogues, instruction books and atlases (King, 1966; Noltenius and Dittrich, 1962). After an examination survey twenty typical micrographs were taken with the EM Philipps 200 and seventeen with the Jeal 100 B.

## **Results**

The death of mice injected with mouldy-hay-extract began at the equivalent of 800 g of the hay powder per 1 kg live body. The corresponding amount by normal-hay-extract injected mice was 1300 g.

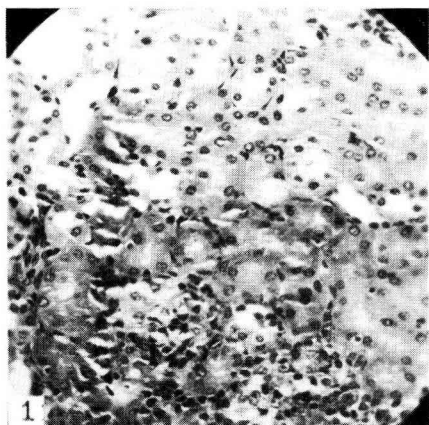
No significant histologic changes were noted in the kidneys and liver of the normal-hay-injected mice (at a level of 400 g of the hay powder per 1 kg live body) except for slight fatty degeneration.

Histological preparations of the mouldy-hay-extract injected mice (same dosage) revealed cloudy swelling in all preparations, fatty and parenchymatic degeneration in the kidney and liver of two thirds of the animals, and general nephrosis to the stage of vacuolisation of the cells in half of the mice. The histopathologic changes as seen in the light microscope are shown in the figures 1, 2 and 3.

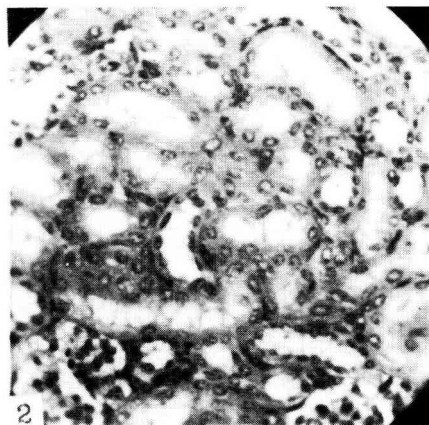
The histopathogenic changes in the kidney tissue of the mice treated with toxin injections as compared with the controls become obvious when micrographs of both sets are laid side by side.

The micrograph in figure 4 shows tissue taken from the vicinity of a proximal convoluted tubule of the kidney of a control mouse. From right to left there appear the basal membrane, the areas of elongated mitochondria,

1 : Final confirmation of the identification was given by the Centraalbureau voor Schimmelcultures, Baarn, Holland.



*Fig. 1* — Cloudy swelling.  $\times 360$ , H and E.



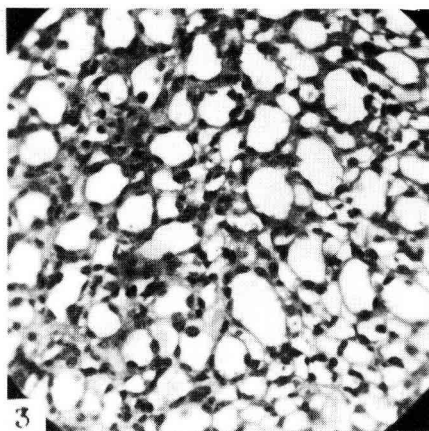
*Fig. 2* — Nephrosis and fatty degeneration.  $\times 360$ , H and E.

and of apical canaliculi, and the fine brush of microvilli plating the lumen. The appearance of a great number vesicles may indicate reabsorption pinocytosis. The regular shape of the mitochondria and their cristae and membranes, as well as the zonal arrangement of microvilli that plate the lumen, is outstanding. The black vesicula in the center of the micrograph is a myelin figure, which is a normal product of cell and membrane destruction.

The second micrograph (fig. 5) is from the same mouse in the same area, but at higher magnification. A part of the nucleus, mitochondria and the pattern of microvilli are shown.

The micrograph in figure 6 is taken from a similar area of the toxin-treated mouse. It shows the bow-shaped basal membrane of the tubuli and above it a layer of elongated mitochondria. The nucleus is situated in the center. In the top part of the micrograph a pattern of microvilli is seen around the lumen. Most of mitochondria are disturbed. Around them lie granular particles, black glycogen globules, and large numbers of vesicles greatly enlarged to the size of mitochondria. All these are symptoms of active phagocytosis and pinocytosis for the defence of the kidney. The structure of the endoplasmatic reticulum and the microvilli is blurred.

The striking changes in the microvilli brush are most marked, when figure 7 is compared with figure 8. The first shows the microvilli



*Fig. 3* — Vacuolisation of the cells.  $\times 360$ , H and E.

brush of a control mouse. The second shows a similar area from a toxin-treated mouse. Here we note marked structural degeneration of the tissue, known in light microscopy as « cloudy swelling ». The walls of the microvilli are thickened, their structure is disturbed and partly destroyed. The advanced stage of the degeneration is shown in figure 9.

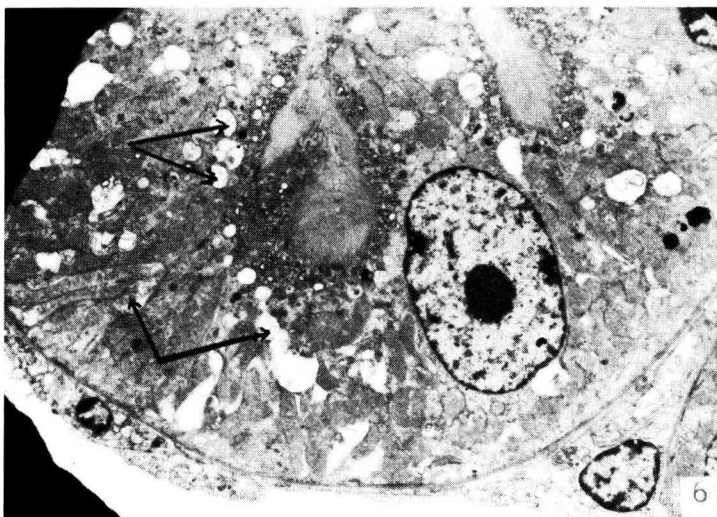
The micrograph in figure 10 shows tissue that has been taken from the area around a



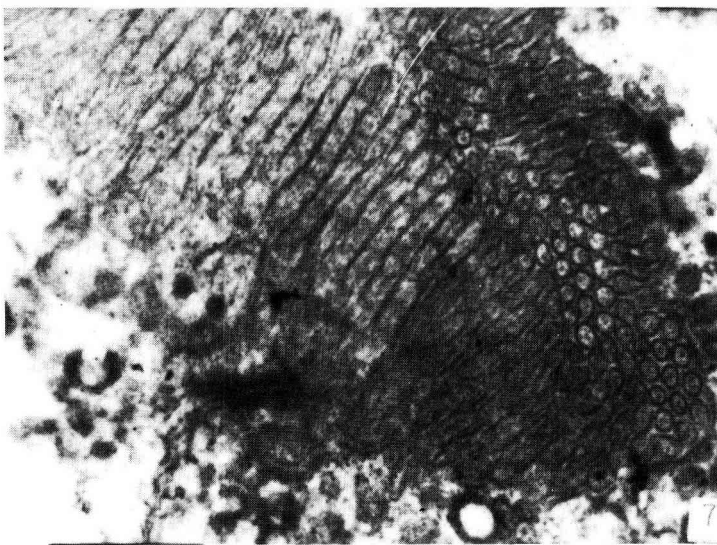
Fig. 4. — Area of proximal convoluted tubule from kidney of control mouse.  $\times 17000$ .



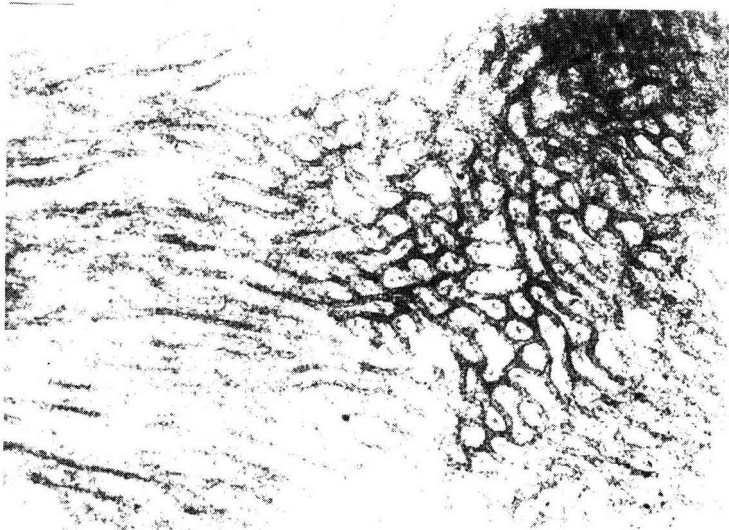
Fig. 5. — Same area enlarged.  $\times 36000$ .



*Fig. 6.* Similar area from toxin treated mouse.  $\times 9000$ .



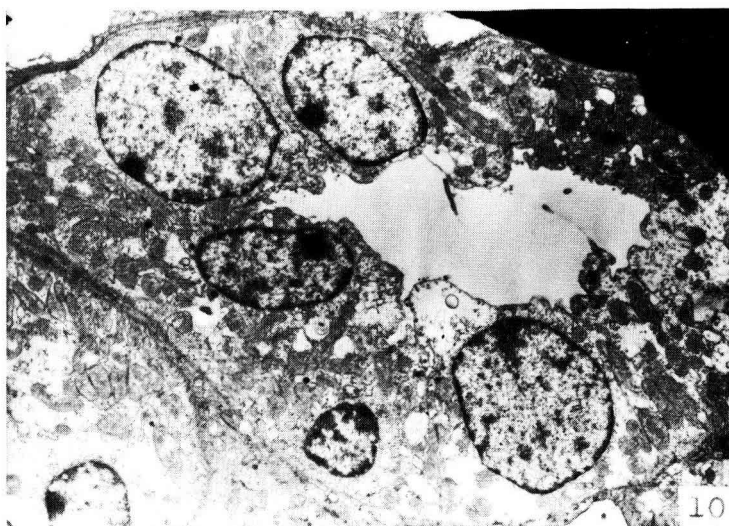
*Fig. 7.* Area of microvilli brush from kidney of control mouse.  $\times 36000$ .



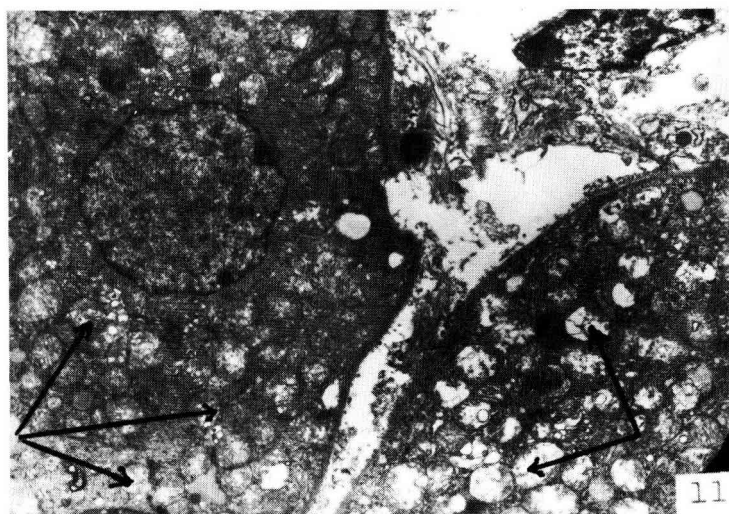
*Fig. 8.* — Similar area of toxin-treated mouse.  $\times 36000$ .



*Fig. 9.* — Similar area of toxin-treated mouse.  $\times 36000$ .



*Fig. 10.* Area of distal convoluted tubule from kidney of control mouse.,  $\times 12000$



*Fig. 11.* Similar area of toxin-treated mouse.  $\times 12000$ .

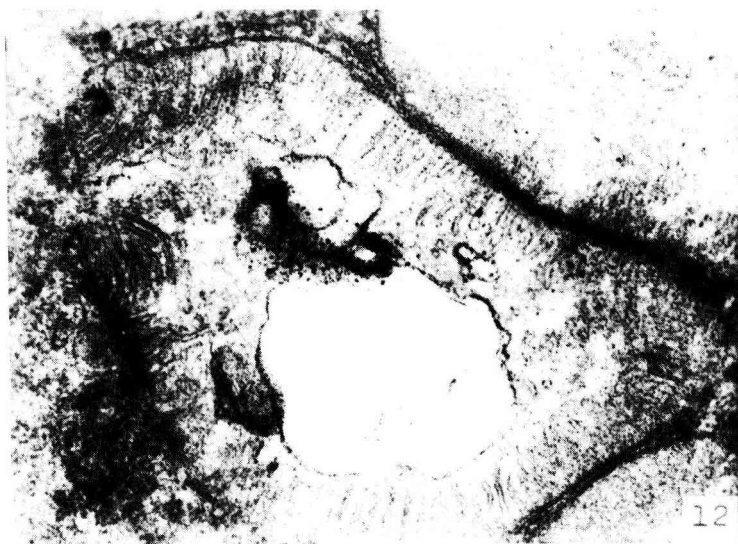


Fig. 12. Same area enlarged. · 36000.

distal convoluted tubule of the kidney of a control mouse. Five nuclei of five separate cells are seen surrounding the central lumen of the tubule for solvent drainage. Several mitochondria, most of them divided into cristae, are also seen. All the items are clear and complete including the enveloping membranes of the nuclei and mitochondria. Also shown are the basal membrane of the tubuli and a large number of secretory vesicles in the neighbourhood of the lumen. This is a typical view of active tissue.

Figure 11 shows a similar area from a toxin-treated mouse. Damage to the mitochondria is apparent. Their shape is destroyed and cristae are absent. The nuclei are still normal. The vesicles appear in large numbers, as often occurs in exaggerated pinocytosis and phagocytosis. This is characteristic of the defence activity of the kidney.

In the last micrograph (fig. 12) a ruptured mitochondrion (caverne) is shown, greatly magnified.

## Discussion

Several histopathological lesion identified

by light microscopy as cloudy swelling, fatty and parenchymatic degeneration and vacuolisation have been more accurately diagnosed by electron microscopy. Pathological changes, such as excessive secretion of glycogen, lysis of membranes and organella structures, increased number of vacuoles and vesicles — have been demonstrated.

The general nephrosis may be caused chiefly by mycotoxins and the vacuolisation of the cells by phenolic compounds, as suggested in an additional experiment (Shadmi *et al.*, 1974) with 24 animals (5 mice, 15 rats, 2 guinea pigs, 2 kids) in which separate subfractions of ether extract were injected.

Other symptoms, such as caryolysis and caryolaxis and many types of infiltration, have not been explained. In spite of many efforts, specific changes in the lysosomes or Golgi apparatus, which are supposed to be symptoms of their antitoxic activity, were not verified. The state of « general nephrosis » or « interstitial nephrosis » seen in the light microscope was not sufficiently defined in the electron microscope. The reason may be that sampling of the tissue was random.

No doubt the light microscope, which

covers a broader field, is able to provide more information for diagnostic purposes. With the electron microscope it is possible to obtain additional specific information on the pathological activity of the cell and pathological changes occurring within it.

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### Summary

A number of histopathological changes, such as cloudy swelling, fatty and parenchymatic degeneration and vacuolisation caused in kidney by mycotoxins from mouldy hay, were examined and studied with the aid of the electron microscope. Changes were observed in mitochondria, membranes, plasma granulation, glycogen secretion, vesiculation and vacuolisation.

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