

Marked morphological and functional changes in the kidneys were found in rats receiving sodium fluoride daily by subcutaneous injection in a dose of 12 mg/kg for 12 weeks.

Chronic fluorosis in man is known as an endemic or occupational disease. In connection with the increasing use of fluorine-containing compounds, elucidation of the pathogenesis of fluorosis is an increasingly urgent task. Fluorides, on entering the body, are partly deposited in the calcified tissues and excreted in the urine or feces. This accordingly points to a lesion of the kidneys as a possible result of the increased intake and elimination of fluorine [5, 10, 11].

Because of the conflicting character of data in the literature on this problem, the kidneys were investigated in rats with chronic sodium fluoride poisoning.

EXPERIMENTAL METHOD

Sodium fluoride in a dose of 12 mg/kg was injected daily subcutaneously for 12 weeks into 90 male albino rats initially weighing 120 ± 20 g. Forty animals were examined for 12 weeks after the end of sodium fluoride administration (the recovery period). The control consisted of another 40 animals kept under the same conditions but not receiving sodium fluoride. The rats were sacrificed by decapitation at different times: 4 h, 2 days, and 1, 2, 4, and 12 weeks after the beginning of poisoning, and also 1, 4, 8, and 12 weeks after the end of sodium fluoride administration. The kidneys were fixed in cold 10% formalin, and pieces of kidney tissue were embedded in paraffin wax. Longitudinal sections were cut through the center of the papilla and stained with hematoxylin-eosin, with Heidenhain's azan, and by the combined Ritter-Oleson method, and also impregnated with silver by Gomori's method. Sections cut on the freezing microtome were stained with Sudan III and Sudan black. Activity of ATPase and alkaline and acid phosphatases was determined histochemically in 60 animals after poisoning for 2 days, and 2, 4, and 12 weeks, and again in the recovery period 1, 4, and 12 weeks after the end of poisoning. In frozen sections ATPase activity was determined by the method of Wachstein and Meisel, acid phosphatase activity by the use of naphthol AS-BS phosphate in incubation fluid at pH 5.2, and alkaline phosphatase by the use of naphthol AS-TR phosphate in incubation fluid at pH 9.0 [9].

EXPERIMENTAL RESULTS

Postmortem examination of rats receiving fluorine for longer than 4 weeks and of rats before the 4th week of the recovery period showed a sharp decrease in the quantity of paranephric adipose cellular tissue. The kidneys were livid or brownish in color and slightly thickened.

The presence of acid mucopolysaccharides (AMP) in the interstitial tissues and their distribution in the papilla of the renal medulla are known to reflect the functional state of the countercurrent concentrating system of the kidneys, responsible for the distal reabsorption of free water [1, 2, 8]. Histological examination of the kidneys of the rats sacrificed 4 h after a single dose of sodium fluoride revealed the almost complete disappearance of reactions for AMP in the middle and distal parts of the papilla (Fig. 1b).

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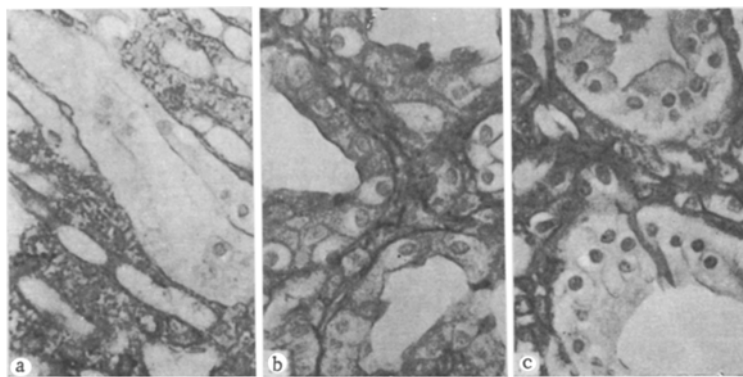


Fig. 1. Content of AMP in interstitial tissue at the apex of the renal papilla of a rat. a) Control; b) absence of AMP (4 h after injection of sodium fluoride); c) low content of AMP (after poisoning for 4 weeks). Ritter-Oleson's stain, 500 \times .

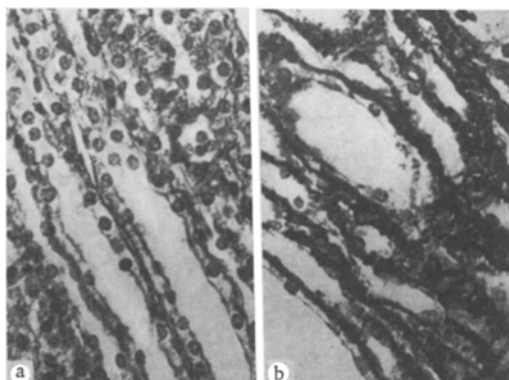


Fig. 2. Argyrophilia of the stroma of a renal papilla: a) Control; b) after poisoning for 12 weeks. Gomori. 500 \times .

The preservation of AMP in the proximal part of the papilla and in the glomeruli indicates the functional significance of this phenomenon, which corresponds to changes observed after prolonged water deprivation or after administration of pituitrin P [3]. Dilatation of the lumen of the capillaries in the distal part of the papilla supplements the histological picture of maximal distal reabsorption. The epithelium of the distal portions of the collecting tubules and the ducts of Bellini were swollen, with a considerable increase in the number of cells with a wide, pale, perinuclear zone. The cell boundaries were not clearly stained by hematoxylin and eosin, but were sufficiently clearly visible after the PAS reaction, and on staining with Sudan black they appeared wide. The apex of the papilla in the experimental animals stained much more weakly with eosin than in the control animals.

In rats sacrificed after administration of sodium fluoride for 2 days, no significant differences from the control animals were found. Persistent changes in the renal medulla

were visible after poisoning for one week. The clearest changes were a marked decrease and, in some cases, the complete absence of AMP in the region of the apex of the papilla (Fig. 1c). The proximal and central accumulation of AMP in the papilla continued at a well-marked level. Some coarseness of the PAS-positive basement membranes was observed at the same time along the whole axis of the papilla. The cytoplasm of the interstitial cells when stained by the PAS method assumed various shades of pink. In every case the AMP of the glomeruli showed no significant difference from that of the control.

In the recovery period, the opposite picture of AMP distribution was observed in the interstitial tissues of the papilla, and it remained until the 4th week. Later, the normal distribution of AMP was restored.

The presence of lipids in the interstitial cells of the papilla is associated in the literature with the concept of a substance of internal secretory nature, possessing local vasodepressor action [6, 7]. In the present experiments, in the first stages of fluorine poisoning, the ordinary distribution of lipid granules was found both in the cells and around the capillaries. However, after the 4th week of poisoning the number of lipid granules was sharply reduced. In the recovery period a gradual accumulation of pseudophilic granules was observed in the interstitial tissues of the papilla.

Histological investigation of ATPase activity in the kidneys showed no significant differences between the experimental and control series either during poisoning or during the recovery period. Alkaline phosphatases activity in the poisoning was appreciably reduced by comparison with the control. In the recovery period the activity of this enzyme in the experimental animals returned to normal after the 4th week. Acid

phosphatase activity also was higher than in the control animals throughout the period of poisoning and until the 4th week of the recovery period in the experimental animals.

The fibrous basis of the interstitial tissue of the papilla, including the basement membranes, showed distinctive changes. In sections stained with Heidenhain's azan, starting with the 4th week of the period of poisoning and at all times of the recovery period studied, the fibrous structures of the papilla were coarser in nature. In sections impregnated with silver by Gomroi's method, a marked increase in the coarseness of the argyrophilic skeleton likewise was found (Fig. 2).

After the 4th week of the period of poisoning, besides degenerative changes in the epithelium of the distal portions of the collecting tubules and the ducts of Bellini, in individual cases clear signs of regeneration were observed in the epithelium, in the form of areas of a double row of epithelial cells containing tiny hyperchromic nuclei. This picture was found until the 4th week of the recovery period. The absence of a constantly visible regenerative process in the renal epithelium in the earlier stages of poisoning can be attributed to the rapid regeneration of the epithelium of the renal tubules, which is usually complete within a few hours [4]. Evidently signs of pathological regeneration could be seen only in the more advanced injuries associated with the cumulative properties of fluorine. Since the epithelial lining of the distal part of the collecting tubules was most severely affected, and it was there that signs of pathological signs of regeneration were visible, it can be postulated that the fluoride concentration in the urine was highest in these parts of the kidney.

In rats investigated in metabolism cages during chronic sodium chloride poisoning, an increase of 2-2.5 times in the diuresis accompanied by a persistent lowering of the specific gravity of the urine were found [5]. The concentration of organic substances in the urine also was high; these were principally nitrogenous substances requiring large volumes of water for their elimination. Under these conditions the water content of the body naturally becomes depleted, and one of the mechanisms of its compensation is an increase in the distal reabsorption of free water.

The histological and histochemical investigations thus showed that chronic fluorine poisoning is accompanied by marked morphological and functional changes in the kidneys.

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