These investigations, revealing differences in the response of different types of apudocytes to ionizing radiation, are evidence of the urgency and necessity of a study of local mechanisms of hormonal homeostasis under extremal influences.

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AUTORADIOGRAPHIC INVESTIGATION OF RNA SYNTHESIS IN KIDNEY TISSUE IN THE EARLY PERIOD OF NECROTIZING NEPHROSIS CAUSED BY MERCURIC CHLORIDE

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KEY WORDS: mercuric chloride nephrosis; RNA synthesis.

Some aspects of the problem of RNA synthesis in kidney tissue in necrotizing nephrosis caused by mercuric chloride have not yet been adequately studied. Considering the importance of such information for the study of the pathogenesis of toxic lesions of the kidneys and for the development of measures aimed at the fastest possible recovery of the damaged organ, it was decided to study, by autoradiography, correlation between the early morphological disturbances and the ability of kidney tissue cells to synthesize RNA.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 150-180 g. The animals were divided into three groups: two rats of group 1 served as the control, animals of groups 2 and 3 (three rats in each group) received mercuric chloride, dissolved in physiological saline, in a dose of 0.5 mg/100 g body weight subcutaneously. The control animals received the equivalent volume of physiological saline. Animals of all groups were given an intraperitoneal injection of ^{3}H-uridine 2 h before sacrifice in a dose of 2 mBq/g body weight (specific radioactivity 370 GBq/liter). Pieces of the kidneys were taken after 24 h from the animals of group 2

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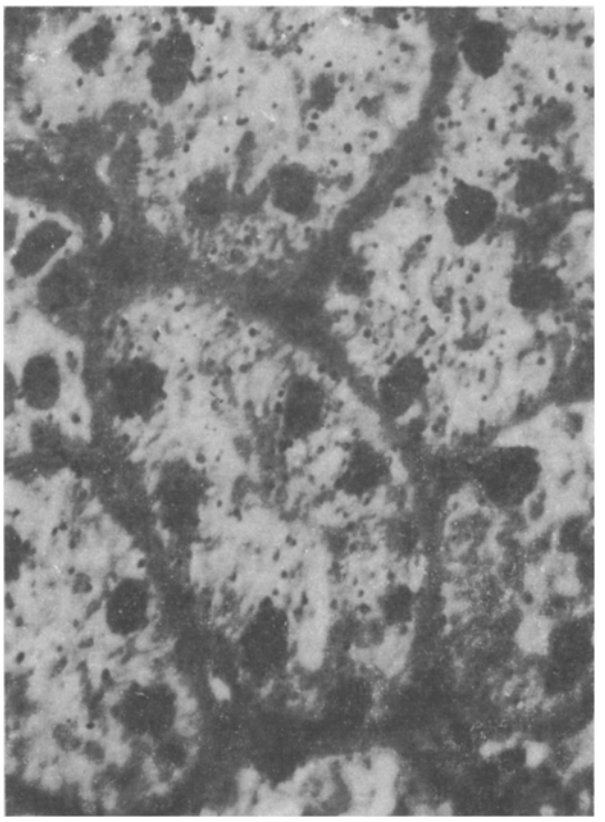

Fig. 1. Localization of grains of silver above nuclei of epithelial cells of proximal tubule of kidney of a control albino rat 2 h after injection of ^{3}H-uridine. Here and in Figs. 2 and 3, semithin section; stained with methylene blue and basic fuchsine; 2000 ×.

and after 48 h from the animals of group 3, killed with ether, and fixed with 3% glutaraldehyde in phosphate buffer and postfixed with 1% osmium tetroxide solution. The tissue was embedded in a mixture of prepolymerized butyl and methyl methacrylate. Semithin serial sections 1 μ thick were coated with type M emulsion, exposed for 14 days, developed, and stained with methylene blue and basic fuchsin [5].

EXPERIMENTAL RESULTS

Analysis of the autoradiographs showed that incorporation of ^{3}H-uridine into intact kidney tissue is heterogeneous in character and depends on the type of cells forming the renal corpuscles, urinary tubules, and structures of the microcirculatory bed and interstitial tissue. Incorporation of ^{3}H-uridine was most intensive into nuclei of the endothelium of the glomerular and peritubular capillaries, nuclei of the podocytes, and nuclei of the interstitial tissue of the renal cortex and medulla. Throughout the extent of the nephron considerable accumulation of ^{3}H-uridine was found in the proximal segments, located in the cortex, with a gradual decrease toward the loop section and a smooth increase up to a high level in the distal convoluted tubule (Fig. 1). A quite high intensity of label was observed throughout the length of the collecting tubules.

The animals receiving mercuric chloride in the dose mentioned above developed anuria after 24 h. The study of autoradiographs obtained from the rats of this group revealed much reduced incorporation of ^{3}H-uridine into nuclei of all types of cells. It must be pointed out, however, that against the background of a general marked decline of ^{3}H-uridine incorporation into the kidney tissue, enlarged epithelial cells with hypertrophied nuclei and nucleoli, above which there was a large number of grains of silver, were found in some damaged urinary tubules and in tubules in which no visible morphological changes could be seen (Fig. 2). Under a magnification of 400, several such cells could be seen in one field of vision, mostly close to the renal corpuscles. Grains of silver in cells of this kind were concentrated

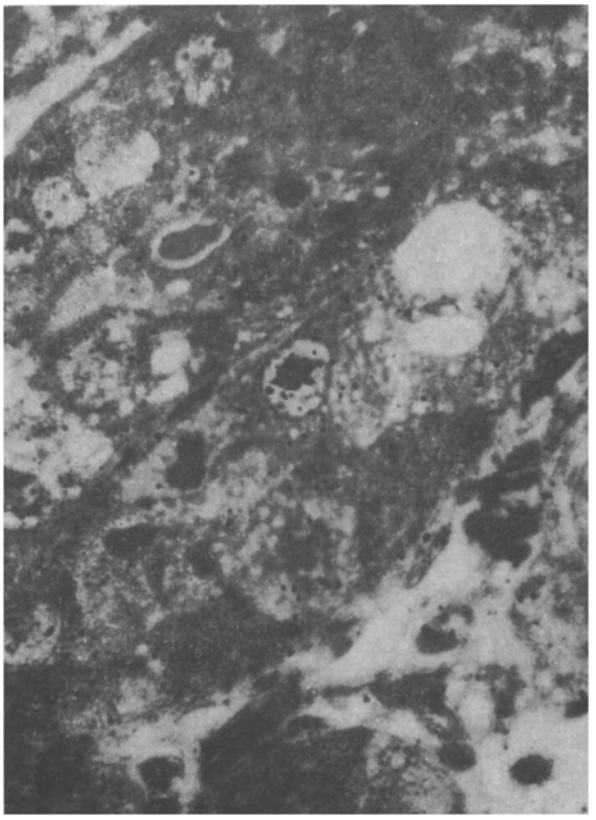

Fig. 2. Intensive incorporation of ^{3}H-uridine into nucleolus of hypertrophied epithelial cell of damaged proximal tubule of rat kidney 24 h after injection of mercuric chloride in a dose of 0.5 mg/100 g.

mainly above the hypertrophied nucleolus, whereas in nuclei of epithelial cells of the proximal part of the nephron of the control animals grains of silver were located above the whole of the nucleoplasm.

In autoradiographs obtained from animals 48 h after injection of mercuric chloride, an increase in the amount of radioactive label was observed in cell nuclei of the renal corpuscles and in epithelial cell nuclei of the urinary tubules, located in the cortex. In the outer medullary layer, where necrotic changes were most marked, an increase in the amount of ^{3}H-uridine label was observed in nuclei of the interstitial tissue. At this period, on the boundary with the zone of necrosis, in intact and partially damaged urinary tubules, but with parts still intact, the number of large epithelial cells with hypertrophied nuclei and intensively labeled nucleoli was considerably increased. These cells were distributed unevenly in the cortex. They were absent in some tubules, whereas in others they were present in small groups.

The fact will be noted that, besides enlarged epithelial cells with hypertrophied nucleus and intensively labeled nucleolus, epithelial cells with nuclei of the normal size or only slightly enlarged, and with intensive labeling, and cells with hypertrophied nuclei and nucleoli with no sign of incorporation of ^{3}H-uridine, were sometimes encountered.

The results are indirect evidence that before mitotic division, epithelial cells of urinary tubules which have suffered a varied degree of damage undergo hypertrophy and synthesize RNA intensively. Since these cells are enlarged in volume, it is difficult to judge from what degree of partial necrosis they began to grow. However, in damaged tubules cells were seen which had lost a considerable volume of their cytoplasm and had a few grains of silver above their nuclei, which were of the normal size, evidence that they preserved their ability to synthesize RNA.

In a detailed study of the preparations the impression was obtained that not only the volume of apical cytoplasm lost, but also changes taking place in the basal part of the cell are very important for the epithelium of the proximal tubules, for cells or layers of cells with

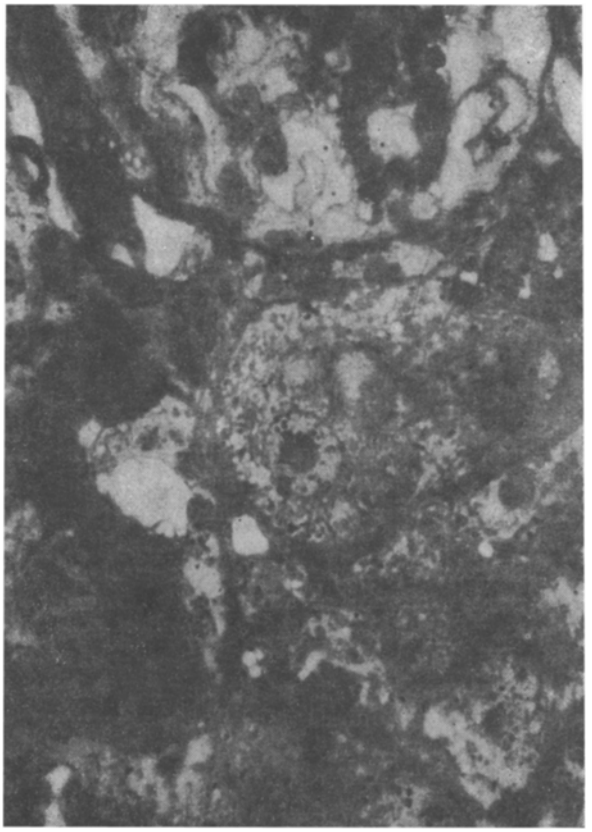

Fig. 3. Cortex of albino rat kidney 48 h after subcutaneous injection of mercuric chloride in a dose of 0.5 mg/100 g. Hypertrophied epithelial cell with intensive ^{3}H-uridine labeling of nucleolus, in the basal part of which there are numerous small vacuoles, can be seen in an injured urinary tubule, close to the renal corpuscle.

well preserved cytoplasm but which have lost their connection with the basement membrane because of intensive vacuolation, may sometimes be seen. Vacuolation was observed in some hypertrophied cells with signs of intensive RNA synthesis (Fig. 3).

The results of this investigation are in agreement with data obtained by other investigators who studied the RNA and DNA content in kidney tissue homogenates from rats with mercuric chloride nephrosis [4]. In the publication cited it was noted that after injection of mercuric chloride intravenously into animals in a dose of 1.5 mg/kg body weight the RNA content in the kidney was reduced during the early necrotic phase, and 24 h after poisoning, but later it started to rise and reached a maximum on the 4th day.

General inhibition of RNA synthesis in kidney tissue cells may perhaps be connected with disturbance of the blood supply and edema of the renal tissue, with consequent metabolic acidification. In dogs, for example, after injection of mercuric chloride in a dose of 3 mg/kg body weight, the renal blood flow was reduced by half within 3 h [6]. We know from data in the literature that metabolic acidification of the pericellular medium may be a factor limiting cell proliferation [3]. The fact that marked destruction of the urinary tubules of the proximal part of the nephron is found 12 h after injection of mercuric chloride into the animals, and that incorporation of ^{3}H-thymidine and mitotic activity reach a maximum on the 3rd day, may perhaps be connected with this effect.

On the basis of the present and previous investigations [1, 2] we can draw the preliminary conclusion that epithelial cells which have undergone a varied degree of partial damage preserve their viability, as is shown by their ability to incorporate ^{3}H-uridine and ^{3}H-thymidine. A certain proportion of damaged cells remains capable of DNA replication, mitosis, and cytokinesis, and it constitutes cellular material capable of covering the denuded basement membrane of some tubules in the zone of necrosis for a certain time. In some cells the

process of cytokinesis is disturbed, as is shown by the appearance of large binuclear cells, and some cells after passing through the S period are held up in the G_2 period because of their inability to go into mitosis, they undergo a varied degree of hypertrophy, accumulate in the urinary tubules, and later, are destroyed after different time intervals.

All these disturbances of the cell cycle can be explained by the primary action of mercury on sulfhydryl groups of nuclear and cytoplasmic proteins, or by their secondary action, as a result of disturbance of the blood supply to the kidney tissue and hypoxia, which is accompanied by activation of endogenous phospholipases and lipid peroxidation. The probability likewise cannot be ruled out that the appearance of hypertrophied cells is compensatory and adaptive in character and is connected with intensification of the functions of regenerating cells while in the G_1 period. This is supported by the fact that more hypertrophied cells are found during the period when anuria gives way to diuresis. Evidently a final decision regarding the period in which the hypertrophied cells will be drawn only after experiments with repeated injections of ^{3}H-thymidine.

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QUANTITATIVE EVALUATION OF CYTOARCHITECTONIC REORGANIZATION OF THE LOCUS COERULEUS INDUCED BY 6-HYDROXYDOPAMINE

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KEY WORDS: brain; neurotoxin 6-hydroxydopamine; degree of homogeneity and isotropism of structures.

The property of the specific neurotoxin 6-hydroxydopamine (6-OHDA) of selectively injuring the catecholaminergic system of the brain is widely used in order to study its structural and functional organization. Experiments with intracisternal injection of 6-OHDA provide the most adequate model of neurological disorders associated with a reduced catecholamine concentration in the brain. When injected in this way 6-OHDA penetrates into the intercellular medium and interacts with catecholamine receptors, so that responses of nerve tissue structures to the initial and gradual damage to the catecholaminergic system can be studied. The main targets for the action of 6-OHDA are dopaminergic structures, but there is evidence that a single injection of 6-OHDA can induce a long-term fall in the noradrenalin level by 70-80% (the main source of the noradrenalin is considered to be the locus coeruleus of the brain stem [2]). We know from postmortem observations that in neurological and mental disorders caused by catecholamine deficiency, the basophilic protein bodies characteristic of neurons of monoamine groups disappear both in the cytoplasm of neurons of the locus coeruleus and in the substantia nigra [7]. Death of neurons of the locus coeruleus has been reported in experiments on rats with the use of 6-OHDA, and in explants of organotypical cultures [3, 5]. Meanwhile other workers found no significant disturbances of the structure of the locus coeruleus under analogous experimental conditions [8]. Thus the data in the literature are scanty and contradictory, yet the obtaining of morphological evidence of structural transformations in the neurons of the locus coeruleus under conditions of injury to the catecholaminergic system is

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