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DESTRUCTIVE AND REGENERATIVE CHANGES IN THE ALBINO RAT KIDNEY DURING MERCURIC CHLORIDE NECROTIZING NEPHROSIS

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Mercuric chloride (corrosive sublimate), which acts as a nucleus for the thiol group, 24 h after introduction into animals gives rise to heterogenous necrotic changes in the epithelial cells of the renal tubules. Despite many histological investigations devoted to the study of regeneration in mercuric chloride necrotizing nephrosis, many disputed and unsolved problems to do with determination of the sources of reparative regeneration of the renal tubular epithelium still remain.

According to some data [5, 6], regeneration of the epithelium of the proximal urinary tubules, damaged by mercuric chloride, takes place by ingrowth of mitotically dividing cells of the uninjured epithelium located distally to the zone of necrosis. According to histological and histoautoradiographic data obtained by other workers [2-4, 8], the source of regeneration of the necrotically changed epithelium of the urinary tubules is solitary cells which remain viable in the zone of necrosis, which resemble epithelial cells of loop segments located below, which later cover the injured areas of the tubule again. On the basis of the results of an extensive study of autopsy material, Permyakov and Zimina [1] postulate that partially injured epithelial cells ("amputant" cells) in the zone of necrosis are able, in the early period of necrosis, to perform the role of primary "patch," protecting the basement membrane of the tubules, and later the set of preserved epithelial cells acts as the source for true proliferation, covering the denuded areas of the basement membrane.

This paper describes the results of a morphological analysis of destructive and regenerative changes observed during a study of serial semithin sections of the kidneys of albino rats with mercuric chloride necrotizing nephrosis.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 160-220 g. The animals were divided into two groups: Six rats of group 1 served as the control, 18 rats of group 2 received a single subcutaneous injection of mercuric chloride in a dose of 0.6 mg/100 g body weight, dissolved in physiological saline.

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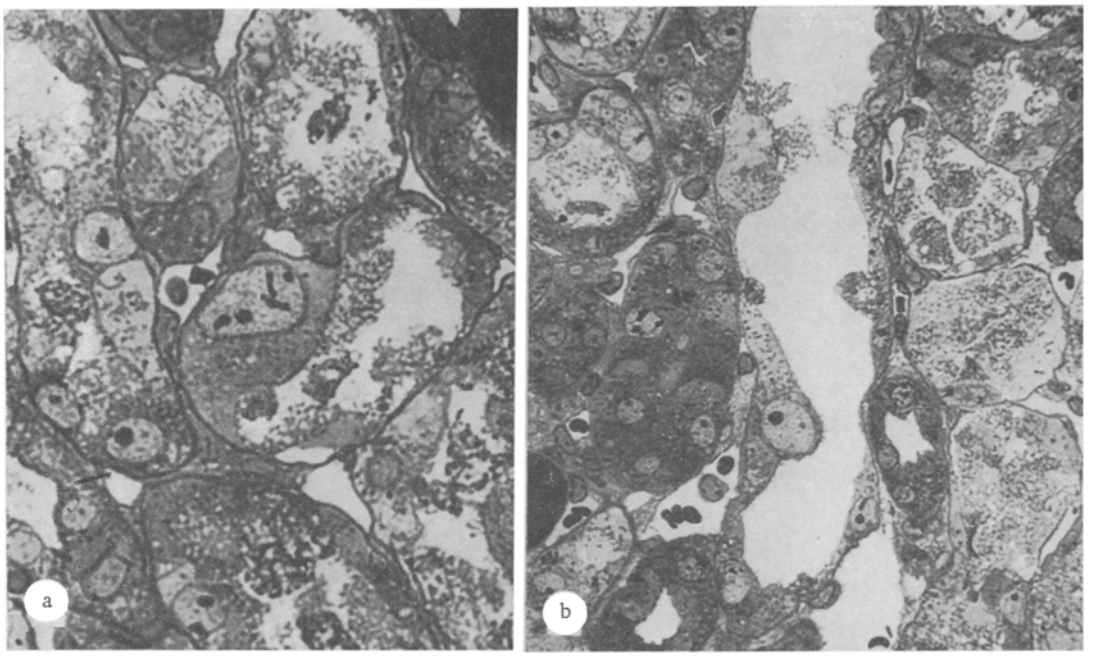

Fig. 1. Inner zone of renal cortex of albino rat 3 days after subcutaneous injection of mercuric chloride in a dose of 0.6 mg/100 g. a) Edematous epithelial cell, without microvilli, containing very large nucleus and many polymorphic nucleoli, is present in lumen of damaged urinary tubule (in center); b) enlarged epithelial cell with large round nucleolus among nephrocytes of urinary tubule (in center) which has undergone partial necrosis (500 ×). Semithin section (0.4 μ). Stained with methylene blue and basic fuchsine.

On the 3rd, 4th, and 5th days after injection of mercuric chloride the animals were killed with ether and pieces of the kidneys removed and fixed in Carnoy's fluid and also in 4% paraformaldehyde solution and 1% glutaraldehyde solution in phosphate buffer (pH 7.4), and postfixed in osmium fixative. The tissue was embedded in a mixture of prepolymerized butyl and methyl esters of methacrylic acid in the ratio of 4:1. Semithin (0.4 μ) serial sections (500) were cut on an ultramicrotome in a plane parallel to the loops of the nephron, polychrome stained with methylene blue and basic fuchsine [7], and studied under a light microscope (objective 40, ocular 15), after which a photoreconstruction was made of the thick and thin segments of the descending parts of the nephron loop. Parathin sections 8 μ thick were stained with hematoxylin and eosin.

EXPERIMENTAL RESULTS

Marked destructive changes were observed 3 days after injection of mercuric chloride in both thick and thin segments of the descending parts of the loop of Henle. The basement membrane of the thin segments of the descending part of the loop of Henle in individual nephrons was completely exposed over a wide area or was covered by single epithelial cells which were preserved. As a rule severe necrotic injury to the 6th segment of the descending part of the loop of Henle, with extensive desquamation of the epithelium, was accompanied by widespread damage to the epithelium of the thin segment of the descending part of the loop. The lumen of these injured thin segments usually was dilated and filled with debris for a considerable distance. In nephrons in which the thin segment was less severely injured, mitotic figures were very frequently found.

In the thick segments of the descending part of the loop of Henle, because of the loss of different volumes of cytoplasm by the epithial cells and disturbance of connection with neighboring cells, the inner surface of the tubules became uneven. The cytoplasm of some nephrocytes, which had undergone partial necrosis, was edematous and contained a few granules, whereas the plasmalemma sometimes formed projections of different shapes into the lumen of the tubule. In regions of the urinary tubules located closer to the renal corpuscle, greatly enlarged (by several times compared with the controls) nuclei, containing one or more large, irregularly shaped nucleoli, intensely stained with basic fuchsine (Fig. 1a), were frequently

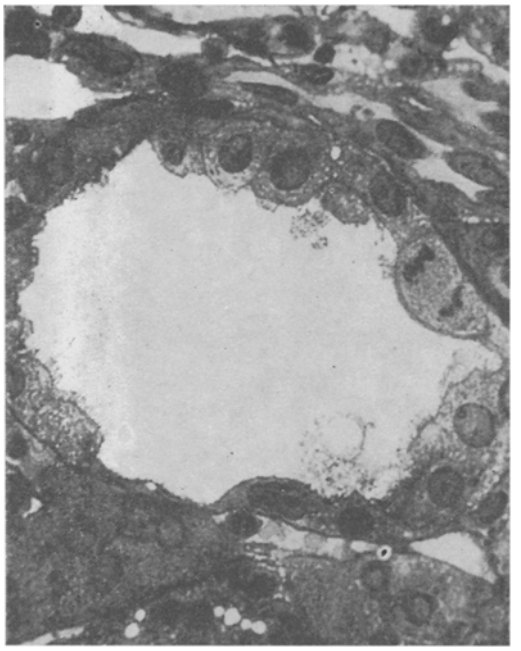

Fig. 2. Inner zone of renal cortex of albino rat 4 days after subcutaneous injection of mercuric chloride in a dose of 0.6 mg/100 g. Nephrocyte in anaphase of mitosis can be seen in a dilated urinary tubule among epithelial cells which have undergone a varied and marked degree of partial necrosis. Semithin section (0.4 μ). Methylene blue and basic fuchsine. 750×.

found at this period in the edematous cytoplasm of the injured nephrocytes. This structure of individual injured nephrocytes evidently reflects their agonal state, for giant nuclei with massive atypical nucleoli can often be seen in cytoplasm undergoing destruction.

In some animals 4 days after injection of mercuric chloride pale vacuoles, varying considerably in number and size, were present in the cytoplasm of epithelial cells of the urinary tubules still preserved after partial necrosis. The formation of multiple vacuoles in the apical part of the cell leads to rupture of the plasmalemma and destruction of the cell. Often numerous tiny vacuoles were formed in the basal part of the cell, causing detachment of the cells from the basement membrane and their desquamation into the lumen of the urinary tubules. This desquamation of epithelial cells may perhaps be due to removal of debris, formed in the early period of mercuric chloride nephrosis, from the tubules and the beginning of passage of urine.

The fact will be noted that this dose of mercuric chloride caused death of almost 60% of the animals on the 4th day. In a study of sections through the kidneys before death of the animals areas of nephrons denuded of their epithelium over a wide area could be seen. In sections through the kidneys of rats still alive after 4 days the basement membranes of the renal tubules were nearly always covered with a layer of greatly flattened epithelial cells. Since it is possible in semithin sections to distinguish quite accurately between these very flattened cells, which have consequently undergone marked partial necrosis, and regenerating cells, this means that the formation of this epithelial lining from accelerated mitotic cell division can be ruled out, and suggests the exceptional importance of epithelium which, although injured, preserves its connection with the basement membrane, for survival of the animal.

Even 5 days after injection of mercuric chloride it was impossible to discover the fate of cells of this very flattened epithelium, covering the basement membrane in some tubules over a wide area.

In tubules in which small areas of greatly flattened epithelium were found, in the adjacent epithelium, which had not undergone any marked degree of partial necrosis, it could

be seen that the cytoplasm of individual cells had become paler and its volume was increased. The presence of large round nucleoli in the nuclei of these cells and of granules in their cytoplasm indicate activation of intracellular synthesis (Fig. 1b). Evidently some of these cells not only remain capable of restoring part of the cytoplasm lost through partial necrosis but also remain capable of mitotic division, for it could frequently be seen that groups of pale cells, sometimes with mitotic figures, replaced areas of greatly flattened, destroyed cells.

Often mitotic figures were found in tubules where epithelial cells were undamaged or where the cells had lost a very small volume of apical cytoplasm, and where regeneration could apparently take place on account of intracellular processes. This hyperplasia was considered to be compensatory, but evidently the possibility could not be ruled out here that this type of response of the epithelium is due to obstruction of the lumen of the urinary tubules by debris, with their consequent gross dilatation (Fig. 2).

The results of this investigation thus indicate that injury to the epithelium of the urinary tubules by mercuric chloride is heterogeneous in depth, and this has a substantial influence on the viability of the animals and on the subsequent process of repair of the damage. Morphological changes in the greatly flattened epithelium were irreversible, and in small areas the epithelium was quickly replaced by proliferating tissue arising from less damaged viable epithelial cells located nearby, whose own structural organization was restored on account of intracellular regenerative processes.

The study of serial semithin sections also showed that regeneration of the tubular epithelium was very complex in character, and it is possible that repeated replacement of the newly formed primary proliferating tissue by more normal nephrocytes could take place, for the function of many cells of the regenerating tissue was disturbed, as shown by the numerous calcified deposits formed in the cell cytoplasm.

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