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CORRELATION BETWEEN INTRACELLULAR AND CELLULAR REGENERATION OF THE RENAL TUBULAR EPITHELIUM OF THE KIDNEY IN NECROTIZING NEPHROSIS

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KEY WORDS: mercuric chloride-induced necrotizing nephrosis; autoradiography; DNA synthesis; intracellular regeneration

Mercuric chloride (corrosive sublimate) has nephrotoxic properties, and on entering the animal or human body it induces necrosis of the tubular epithelium, accompanied by a varied degree of acute renal failure. This model of toxic necrotizing nephrosis has attracted close attention in connection with the study of the mechanism of action of mercury salts and the principles governing restoration of the structure and function of the renal tubular epithelium since an article was published in 1860 by Pavy [10] describing the effect of mercury on animals, and the place of damage to the renal epithelium was established in rats, rabbits, and dogs [12].

Investigations using transmission and scanning microscopy have revealed the early ultrastructural changes of destructive and regenerative character in the tubular epithelium of the proximal part of the nephron [3-9, 11] and showed that undamaged epitheliocytes may be the source of the proliferating tissue which covers the denuded areas of the basement membrane. Since damaged epithelial cells may also be the source of regeneration of the tubular epithelium [2], the question arises: does intracellular regeneration of the damaged cells take place in mercuric chloride necrotizing nephrosis (MCNN) and, if it does, at what stage of the life cycle does this occur, and what is its connection with the cellular form of regeneration.

In the present investigation, by using information obtained by light and electron autoradiography, an attempt was made to find the answer to the above questions.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 170-210 g. The experimental animals were given a subcutaneous injection of mercuric chloride in a dose of 0.4 mg/100 g body weight, followed 72 h later by injection of ^{3}H-thymidine with specific

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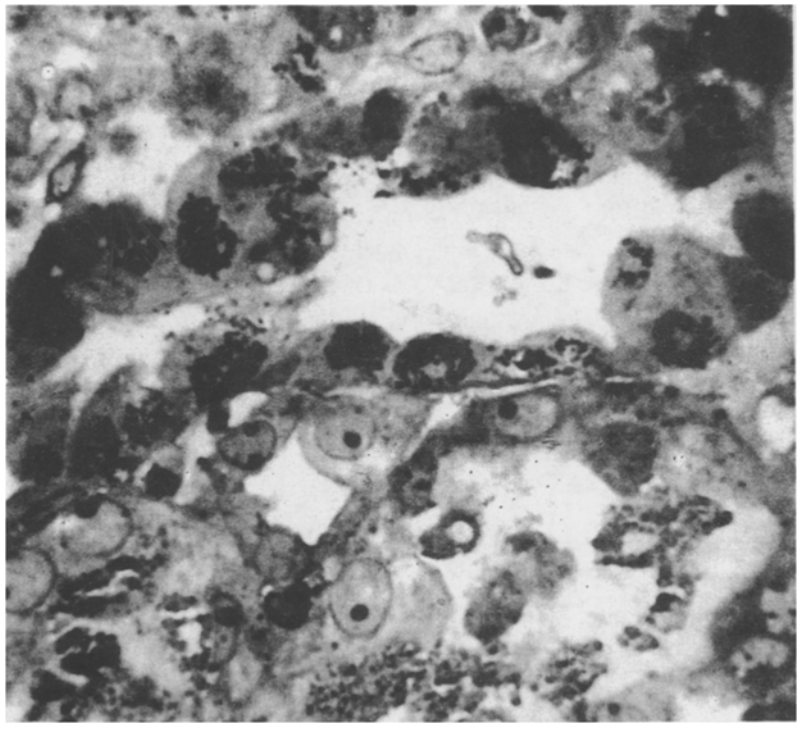

Fig. 1. Cortical substance of albino rat kidney 72 h after subcutaneous injection of mercuric chloride, long-term injection of ^{3}H-thymidine. Partially damaged epithelial cells of the urinary tubule, incorporating ^{3}H-thymidine, can be seen. Semithin section 0.5 μm thick. 1200×.

radioactivity of 207 GBq/liter in a dose of 10 μCi/g body weight. Pieces of kidney were removed from the animals, killed with ether, 1 h after the injection of ^{3}H-thymidine, and specimens were prepared by the usual method for electron autoradiography. For light autoradiography the control and experimental animals were given injections of ^{3}H-thymidine in a dose of 0.5 μCi/g body weight at intervals of 6 h round the clock. The experimental animals of group 1 were killed 72 h after injection of mercuric chloride and the control animals and the experimental animals of group 2 were killed after 6 days. Semithin sections 0.5 μm thick were cut from blocks prepared for investigation under the electron microscope, coated with type M photographic emulsion, exposed for 3 days, and developed, and stained with methylene blue and basic fuchsine.

EXPERIMENTAL RESULTS

Analysis of autoradiographs of the semithin sections showed that labeled nuclei (one or two per field of vision under magnification of 400 times) were found in the control animals after prolonged (6 days) injections of ^{3}H-thymidine, and mainly in cells of the proximal part of the nephron.

In the experimental animals 72 h after injection of mercuric chloride, during long-term labeling incorporation of ^{3}H-thymidine into nuclei of epithelial cells which had undergone partial necrosis with a varied degree of severity was observed in the damaged segments of the proximal part of the nephron. The number of labeled nuclei in the tubules varied considerably depending on the level and degree of damage to the nephron. In less damaged areas of the tubules, located closer to the renal corpuscle, mainly nuclei incorporating ^{3}H-thymidine were found. Incorporation of ^{3}H-thymidine into damaged cells of the urinary tubules during this period was mosaic in character. Tubules cut transversely or obliquely, with intensive incorporation of ^{3}H-thymidine into nearly all the damaged epithelial cells could often be seen in the preparations, together with tubules with the same degree of damage in which the label either was absent or was present in only a small number of cells (Fig. 1). This is evidence that the proliferation process begins at different times in different tubules. In the more distal zones of the proximal part of the nephron labeled nuclei were found much less frequently because of the greater degree of partial necrosis of the epithelial cells and accumulation of solid debris in the lumen of the tubules, where it evidently prevents regeneration of the cells. On the 6th day of prolonged administration of ^{3}H-thymidine, a marked increase in the number of labeled nuclei was found in the renal

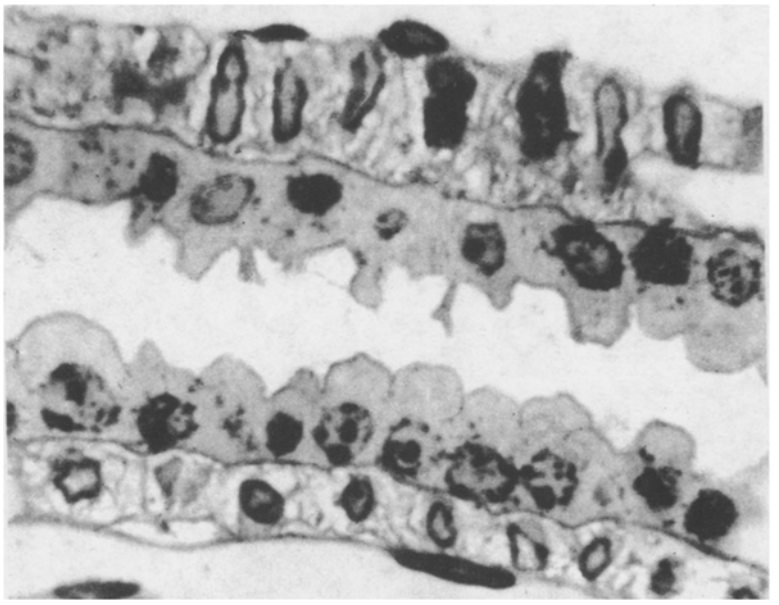

Fig. 2. Inner zone of medulla of albino rat kidney 6 days after injection of mercuric chloride, long-term injection of ^{3}H-thymidine. DNA synthesis in epithelial cells of collecting tubule. Semithin section 0.5-μm-thick. 1200×.

tissue of the experimental animals. In areas where regeneration of the tubules took place the percentage of labeled nuclei in the epithelium reached almost 100. Incidentally, during this period the number of labeled nuclei also increased in undamaged segments of the nephron. Some increase in the number of labeled nuclei compared with the control was discovered on the 3rd day after injury, but it became particularly marked on the 6th day. Grains of silver were often found above the nuclei of the epithelial cells of the cervical portion of the nephron, the epithelium of the capsule, the collecting tubules, the thin portion of the loop of Henle, and the distal tubule (Fig. 2). Cells of the interstitial tissue, located near the renal corpuscles, around the blood vessels and tubules of the cortical substance, and especially in the outer zone of the medulla, incorporated thymidine intensively.

The electron-autoradiographic investigation revealed some differences in the organization of the partially damaged epithelial cells which preserved their ability to synthesize DNA. The S-period of the epithelial cells of the proximal tubules lasted about 9 h, and depending on the time of their stay in the synthetic period, the ultrastructural organization of the damaged cells and localization of grains of silver above their nuclei changed. Considerable damage to the cells was accompanied usually by loss of the nucleolonemal structure of the nucleoli, their condensation, and reduction of their size. A few mitochondria and lysosomes were found in the cytoplasm of these cells and marked destruction of the protein-synthesizing apparatus was noted. Later, in the damaged cells the dimension of the nuclei were increased, but in the condensed nucleoli elements of the nucleolonemal structure began to be apparent. Nuclei containing such nucleoli incorporated ^{3}H-thymidine and grains of silver were found not only above the nucleoplasm, but also frequently above and close to the nucleoli. The nuclei of undamaged epithelial cells contained usually one or, less frequently, two nucleoli. Often as many as three or four nucleoli could be seen in cells which had lost a large volume of cytoplasm.

With development of the nucleolonema the nucleoli increased in size, the quantity of the granular component in them increased, and often they were in close contact with the nuclear membrane. In nuclei with large nucleoli, having a clearly distinguishable nucleolonemal structure, many grains of silver were seen to be concentrated only above the nucleoplasm. These cells were enlarged and many free ribosomes, polysomes, and elements of the rough endoplasmic reticulum were found in their cytoplasm (Fig. 3).

Cells of the type just described are particularly interesting in connection with the theme of this paper. In a previous autoradiographic study of RNA synthesis the present writers [1] showed that cells in damaged tubules with an enlarged nucleus and nucleoli synthesize RNA intensively, especially in the nucleoli. Intensification of RNA synthesis in the nucleolus is evidence of restoration and hyperplasia of the protein-synthesizing apparatus of the cell (the ribosome). It is important to note that intensi-

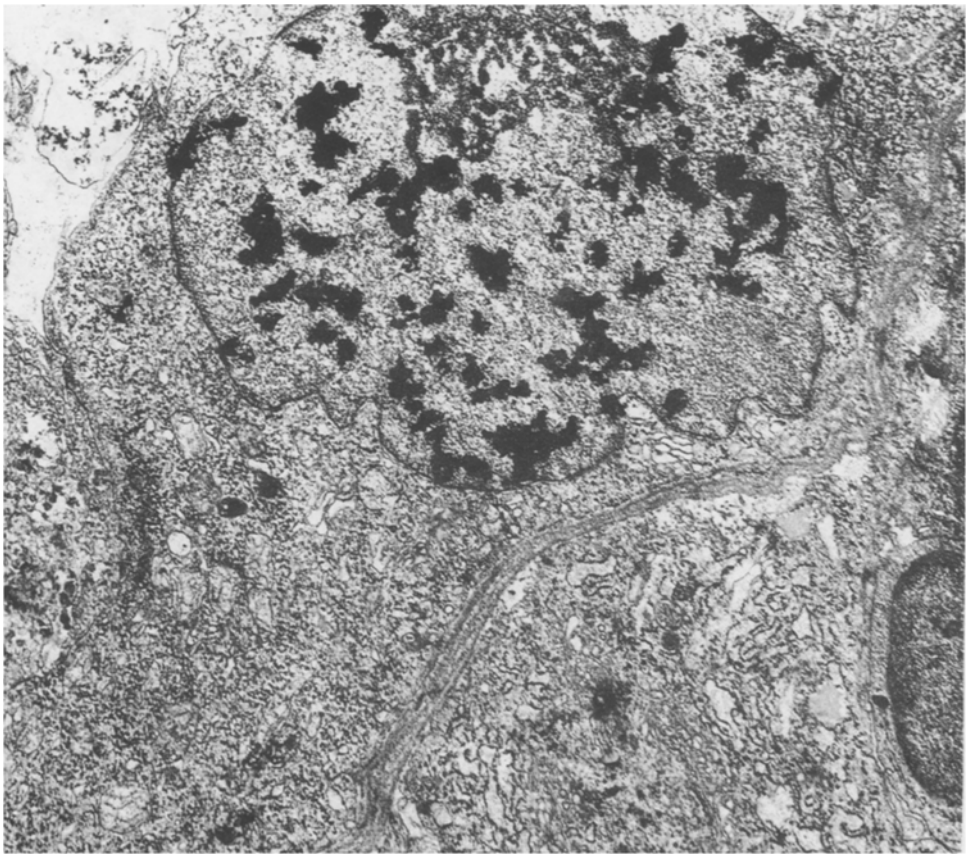

Fig. 3. Proximal part of urinary tubule of albino rat kidney 72 h after injection of mercuric chloride in a dose of 0.4 mg/100 g; pulse-labeled with ^{3}H-thymidine. Many free ribosomes can be seen in the cytoplasm of an epithelial cell which has undergone marked partial necrosis. Grains of silver are distributed throughout the nucleoplasm. The nucleolonemal nucleolus is free from grains of silver. 26,000×.

fication of RNA synthesis in cells with hypertrophied nucleus and nucleoli could be detected as early as 24 h after injection of mercuric chloride, increasing appreciably until 48 h. On the other hand, DNA synthesis was found in these cells after 72 h.

In cells of this morphological type definite correlation between the intracellular and cellular forms of regeneration could thus be identified. The typical response of intracellular regeneration, namely an increase in the number of ribosomes, developed during the 1st and 2nd days immediately after injury, and most probably has a dual biological meaning: it helps to restore the normal function of the damaged cell and organ as a whole and promotes production of proteins of chormatin and of the cytoskeleton that are essential for the normal course of DNA replication and cell division. The cellular form of regeneration, expressed as DNA replication, takes place after the intracellular form and creates the material basis (increases the number of genes in the tissue) for long-term compensation of the damage caused by the pathogenic agent.

For the damaged cell to survive successfully and to subsequently divide, it must evidently preserve a certain minimal volume of cytoplasm with part of the protein-synthesizing apparatus to carry out the earliest stages of proliferation and, possibly, reparative DNA synthesis.

Thus in MCNN epithelial cells which, as a result of partial necrosis, have lost a considerable part of their apical cytoplasm, remain viable and, when stimulated by a certain factor, commence proliferation. Intracellular regeneration develops before cell proliferation and determines the outcome of the pathological process in the time immediately after injury. It must be emphasized that intracellular regenerative processes are closely interconnected with cell division and are aimed at eliminating disturbances which have arisen and which prevent the entry of the cell into mitosis.

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