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AUTORADIOGRAPHIC STUDY OF DNA SYNTHESIS IN RENAL TUBULAR EPITHELIAL CELLS OF ALBINO RATS WITH MERCURIC CHLORIDE NEPHROSIS

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UDC 616.61-002.4-099-092.9-07:616.612-008.939.633.2-073.916

KEY WORDS: mercuric chloride nephrosis; DNA synthesis.

Regeneration of the epithelium of the proximal convoluted renal tubules after damage by mercuric chloride ($HgCl_2$) has now been studied adequately by light-optical methods [3, 4, 7, 9]. Histological investigations have shown that the scale and volume of necrotic changes in the epithelium of the urinary tubules depend on the dose and mode of administration of $HgCl_2$ to the animals. A small dose of $HgCl_2$ causes loss of microvilli and of small areas of apical cytoplasm by the epithelial cells of the straight part of the proximal renal tubule. After injection of $HgCl_2$ into the animals in a dose of 0.4 mg/100 g body weight or more, partial necrosis of the nephrocytes of many urinary tubules is severe in character and is accompanied by sequestration of large volumes of cell cytoplasm into the lumen of the tubules.

Data on the character of reproduction of the renal epithelium, damaged by $HgCl_2$, have been obtained by ^3H-thymidine autoradiography [5, 6, 8]. The use of this method has shown that damaged epithelial cells, preserved in the zone of necrosis, can synthesize DNA, and the duration of periods of the cell cycle of regenerating nephrocytes has been determined. The duration of the cell cycle was found to be 14 h, the period of DNA synthesis 9 h, the presynthetic period 3 h 45 min, and the postsynthetic period 45 min.

In $HgCl_2$ nephrosis the renal tubules are filled with debris, which disturbs the flow of urine and causes dilatation of the lumen of the tubules and marked flattening of damaged epithelial cells, undergoing partial necrosis. The ability of these cells to regain their normal structure and, later, their specific function is a problem of great interest, which is not yet settled.

Since autoradiography with paraffin sections has inadequate resolving power for the detailed study of the structure of damaged nephrocytes and the character of distribution of radioactive label in them, in the investigation described below, to evaluate the structure and DNA-synthetic activity of epithelial cells damaged by $HgCl_2$ it was decided to use semithin (0.5 μ) sections, cut from blocks embedded for electron microscopy.

EXPERIMENTAL METHOD

Noninbred male rats (6 animals) weighing 170-210 g were given a subcutaneous injection of $HgCl_2$ in a dose of 0.5 mg/100 g body weight, and 72 h later an intraperitoneal injection

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(Presented by Academician of the Academy of Medical Sciences of the USSR D. S. Sarkisov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 100, No. 11, pp. 626-629, November, 1985. Original article submitted March 21, 1985.

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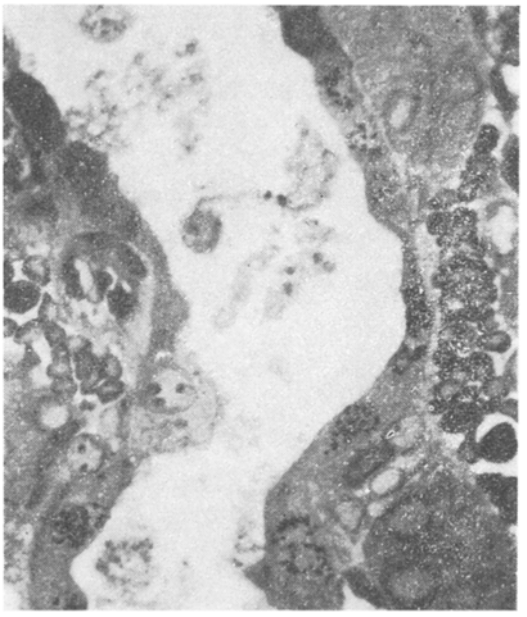

Fig. 1. Uniform distribution of grains of silver above nuclei of nephrocytes which had undergone an equal degree of partial necrosis of apical zones of their cytoplasm 72 h after subcutaneous injection of $HgCl_2$ (900×).

of ^{3}H-thymidine with specific radioactivity of 24 Ci/mmole, in a dose of 10 μCi/g body weight. The animals were killed with ether, and pieces of kidney taken from them 1 h after injection of ^{3}H-thymidine and fixed in 1% solution of OsO_4 in phosphate buffer (pH 7.4). The tissue was embedded in a mixture of prepolymerized butyl and methyl esters of methacrylic acid in the ratio of 4:1. Semithin sections 0.5 μ thick were cut on an ultramicrotome, coated with type M emulsion, exposed for 4 days, developed, and stained with methylene blue and basic fuchsine [10].

EXPERIMENTAL RESULTS

Analysis of the autoradiographs showed that 72 h after injection of $HgCl_2$, nephrocyte nuclei labeled with ^{3}H-thymidine were found in damaged segments of the proximal tubule of the nephron much more often than in intact segments. All the different patterns of distribution of the pulsed thymidine label in nuclei which preserved their normal structure and in nephrocytes with necrotic changes induced by $HgCl_2$ could be divided into three types: massive accumulation of grains of silver over the whole nucleus, a relatively uniform distribution of a few grains of silver over the karyoplasm with a higher concentration above the nucleoli, and concentration of grains of silver in the peripheral zone of the nucleus, where heterochromatin is localized. Marked heterogeneity of distribution of the thymidine label was evidently due to absence of synchronization of DNA replication in nephrocytes damaged to different degrees. This is shown by the fact that in tubules in which there were nephrocytes that had undergone an equal degree of partial necrosis, the distribution of grains of silver indicated roughly equal passage through the synthetic period by the cells (Fig. 1). Often in tubules in which different areas of the apical zones of cytoplasm had been lost by the epithelial cells, nephrocytes in different stages of the synthetic period and different phases of mitosis could be seen.

At this time, incidentally, the pulsed thymidine label could be observed in some nephrocytes which, even though severely damaged, still possessed a small volume of cytoplasm around the nucleus (Fig. 2). As a rule in such nephrocytes the grains of silver were distributed throughout the karyoplasm. We know from the literature [2] that DNA replication initially takes place comparatively uniformly throughout the volume of the nucleus, and localization of the label near the nuclear membrane is observed at the end of the S-period; this is connected with reproduction of heterochromatin, located near the nuclear membrane. Our observations show that the volume of cytoplasm was considerably increased in those nephrocytes in which the thymidine label was concentrated mainly in the peripheral zone of the nucleus (Fig. 3). This may be because reparative processes, leading to restoration of the nor-

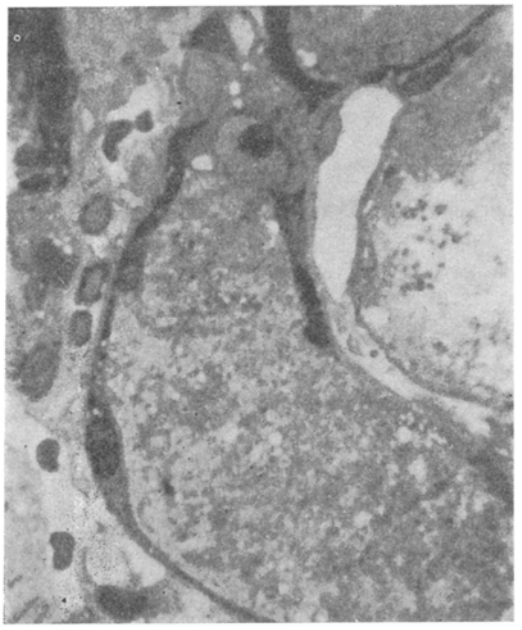

Fig. 2

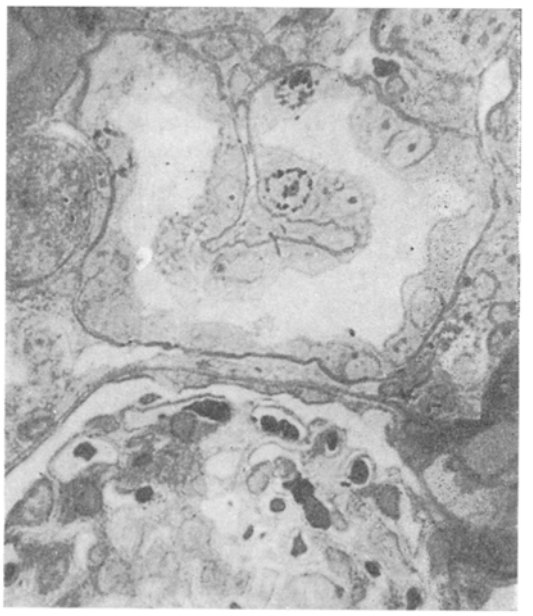

Fig. 3

Fig. 2. Localization of grains of silver above nuclei of two nephrocytes, which have been preserved after losing a large part of their cytoplasm, in a dilated segment of a urinary tubules, filled with debris, 72 h after subcutaneous injection of $HgCl_2$ (1050×).

Fig. 3. Concentration of grains of silver mainly in peripheral zone of nuclei (location of heterochromatin) of regenerating nephrocytes 72 h after subcutaneous injection of $HgCl_2$ (750×).

mal nucleo-cytoplasmic ratio, take place in the cytoplasm of damaged nephrocytes during the S period. A characteristic feature distinguishing necrotic nephrosis due to $HgCl_2$ is the appearance of nephrocytes in the necrotic zone of the kidney with an enlarged cytoplasm and with a nucleus several times larger in size than normal nuclei. The discovery of large, and also of binuclear, cells in some damaged urinary tubules is evidence that they appear, not as the result of intracellular edema (although this does occur in some cells), but as a result of true hypertrophy of the nephrocytes.

It follows from the results of this investigation that many nephrocytes of the urinary tubules which have undergone partial necrosis still remain capable of DNA replication. According to data in the literature [1], the fate of cells commencing the S-period may be to end in complete or polyploidizing mitosis. In other words, under the conditions of necrotic nephrosis induced by $HgCl_2$ damaged epithelial cells can be restored through intracellular regeneration.

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