ELECTRON-AUTORADIOGRAPHIC STUDY OF RNA SYNTHESIS IN EPITHELIAL CELLS OF THE RENAL TUBULES OF ALBINO RATS POISONED WITH MERCURIC CHLORIDE

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Repair of injuries is a property which maintains the resistance of living systems to the action of factors of an injurious character, and can be realized in the form of cellular and intracellular regeneration. Many investigations have now been undertaken to study the cellular form of regeneration of renal tissue after exposure to various influences and in pathological states. Some workers have stated that the epithelial cells of the renal tubules, after undergoing a certain degree of partial necrosis, can undergo mitotic division [3, 5, 6, 9]. The question accordingly has arisen of whether repair processes in a damaged cell take place before its division, and if so, what are these processes, and what is the connection between repair processes and the ability of cells to synthesize DNA? In an earlier electron-autoradiographic investigation [1] it was shown that in mercuric chloride nephrosis the cytoplasm of partially damaged epithelial cells of the renal tubules, but preserving their ability to synthesize RNA, contains many ribosomes. Considering the fact that synthesis and processing of ribosomal RNA and of preribosomes take place in the nucleolus, it was decided to study ultrastructural changes and behavior of RNA synthesis in nucleoli of renal tubule cells in the early period of mercuric chloride nephrosis.

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, whereas animals of groups 2 and 3 (three rats in each group) were given a subcutaneous injection of mercuric chloride, dissolved in physiological saline, in a dose of 0.4 mg/100 g body weight. The control animals received an injection of the same volume of physiological saline. An intravenous injection of [H-uridine with specific activity of 370 GBq/liter, in a dose of 2 mBq/g body weight, was given to the animals of all groups 2 h before sacrifice. The animals of group 2, 24 h after injection of mercuric chloride, and animals of group, 3, 48 h after injection, were killed with ether and pieces of the kidneys were removed and fixed in 3% glutaraldehyde in phosphate buffer, and postfixed with 1% osmium tetroxide solution. The tissue was embedded in a mixture of polystyrene and butylmethacrylate. Ultrathin sections were coated with type M emulsion, exposed for 30 days, stained, and studied in the electron microscope.

EXPERIMENTAL RESULTS

Electron-microscopic study of the autoradiographs showed that the epithelial cells of the proximal tubules of the control animals incorporated the RNA precursor, ^{3}H-uridine, intensively; the intensity of incorporation gradually diminished from segment 1 to segment 3. Most of the ^{3}H-uridine label (grains of silver) was located above the nucleolus and nucleoplasm, but in most cells, approximately half of the label was located above the cytoplasm, at various distances away from the nucleus (Fig. 1). Sometimes cells were seen in which grains of silver located in the cytoplasm were concentrated mainly near the nucleus.

Interphase nuclei of the epithelial cells of the proximal tubule contained most frequently one nucleolus, located centrally or near the nuclear membrane, although occasionally cells with two nucleoli also were seen. The nucleolonemal structure of the nucleolus was indistinct, but the fibrillar and granular components were readily distinguishable. The fibrillar centers

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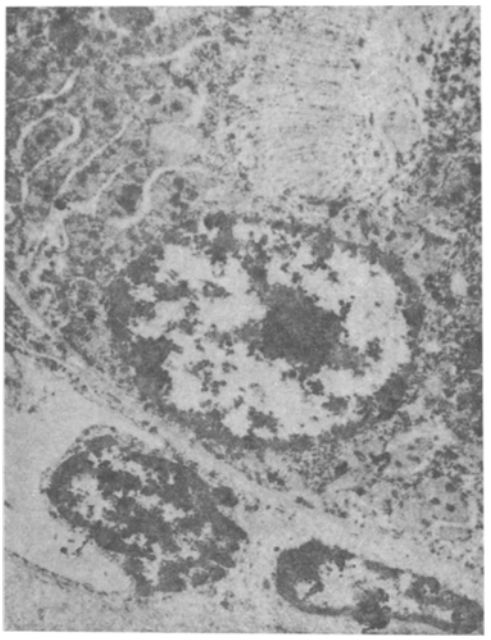

Fig. 1. Autoradiograph of ultrathin section through epithelial cell of proximal renal tubule of control rat, labeled with ^{3}H-uridine. Active synthesis of RNA (grains of silver) observed in nucleolus. Weaker uridine labeling also found above nucleoplasm and cytoplasm. 8000 ×.

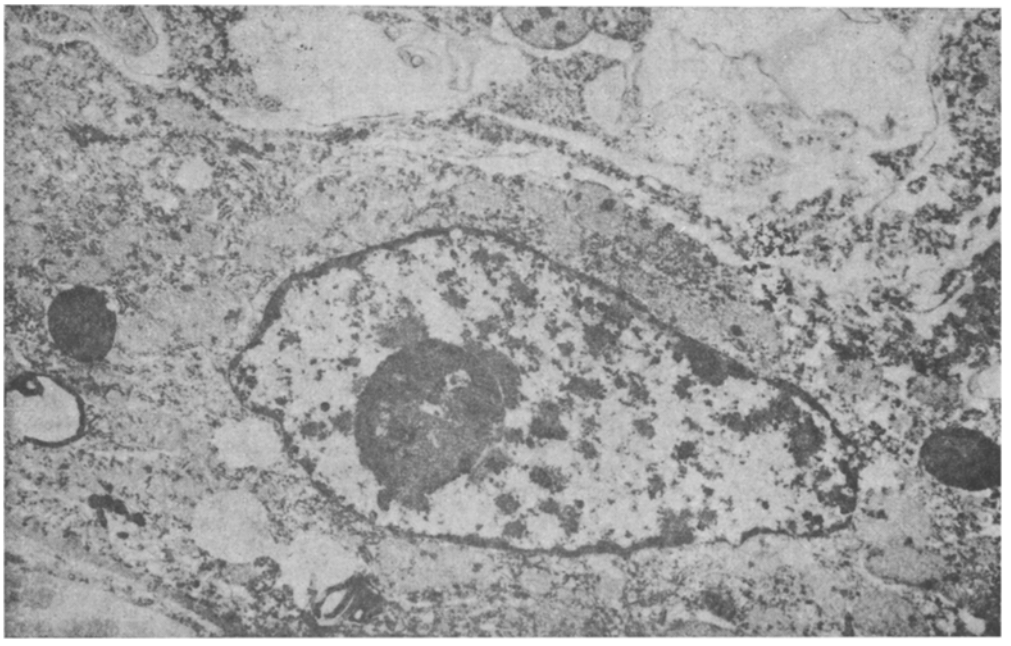

Fig. 2. Autoradiograph of ultrathin section of epithelial cell of proximal renal tubule of a rat labeled with ^{3}H-uridine 24 h after subcutaneous injection of mercuric chloride in a dose of 0.4 mg/0.1 kg. Transition from peripheral chromatin to the diffuse form and suppression of RNA synthesis can be observed in a cell which has lost part of its apical cytoplasm. Perinucleolar heterochromatin partially preserved. 12,000 ×.

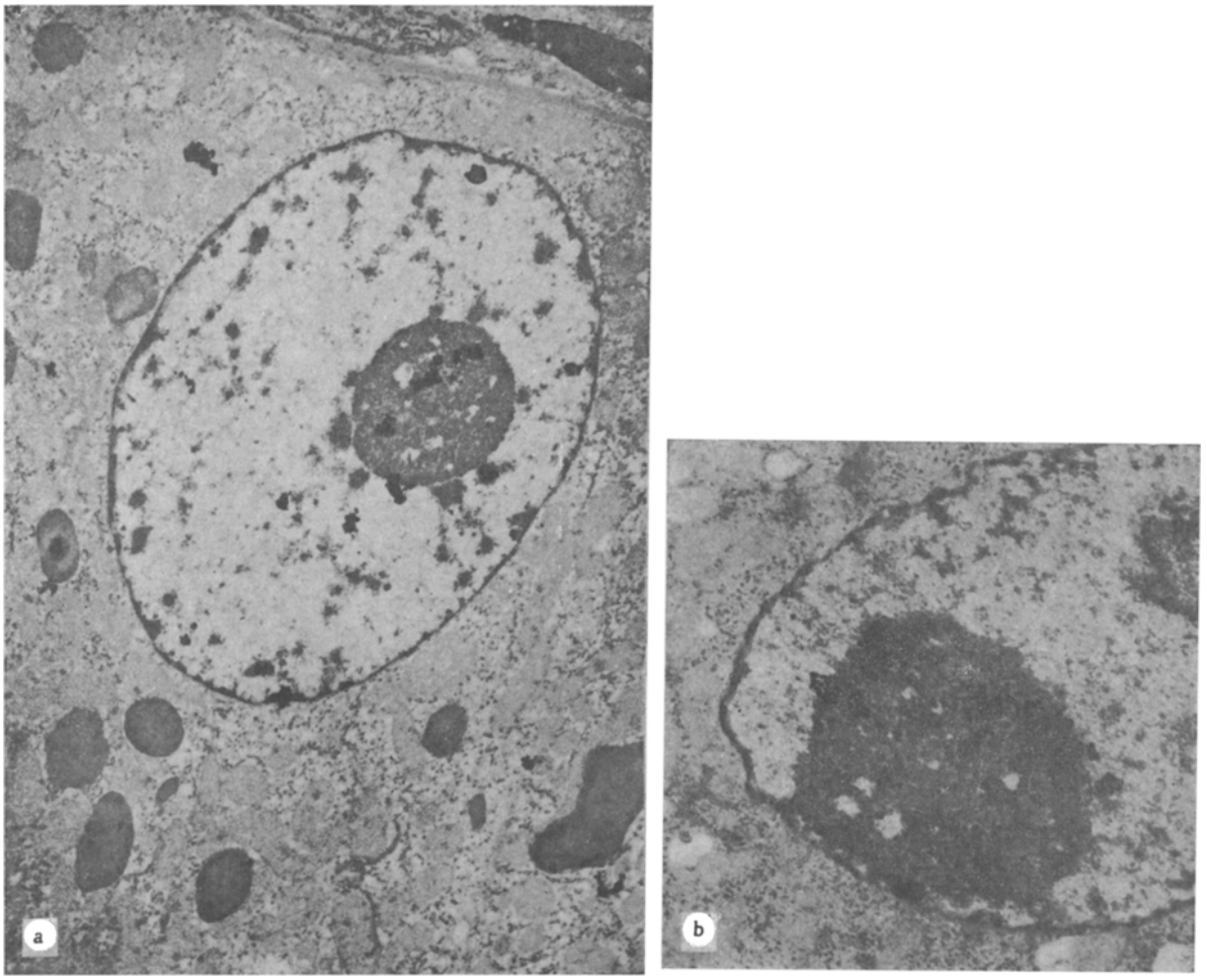

Fig. 3. Autoradiograph of ultrathin section through intact region of proximal tubule of a rat labeled with ^{3}H-uridine, 48 h after subcutaneous injection of mercuric chloride in a dose of 0.4 mg/0.1 kg. a) Enlarged nucleus with decondensed peripheral and perinucleolar heterochromatin contains a nucleolus consisting of the fibrillar component; solitary silver grains above the nucleolus indicate inhibition of RNA synthesis. 16,000 ×; b) numerous silver grains located mainly above large nucleolus, containing granular component, are evidence of activation of RNA synthesis in the epithelial cell. Much of the nuclear heterochromatin is in the diffuse form. 18,000 ×.

were small and few in number. As a rule the nucleoli were surrounded over a wide area by perinucleolar heterochromatin, which in some areas joined the well defined peripheral heterochromatin. The number of heterochromatin islets in the nuclei varied considerably.

In autoradiographs obtained from animals killed 24 h after injection of mercuric chloride a sharp decline in the intensity of ^{3}H-uridine incorporation into the epithelial cells of the proximal tubules was observed. Considerable ultrastructural changes were seen in the nuclei of epithelial cells located on the boundary with the injured part of the nephron, and which had undergone marked partial necrosis. The nuclei were enlarged, their peripheral heterochromatin was greatly decondensed, the chromatin islets reduced in size, and the nucleoplasm became paler. Degranulation and a varied degree of decondensation (sometimes complete) of the perinucleolar heterochromatin could be observed in the nucleoli. In many injured cells the nucleoli were represented by a fibrillar component only (Fig. 2). The few grains of silver above the nucleoli and nucleoplasm of such cells was evidence of suppression of RNA synthesis.

Similar changes in the nucleus could actually be seen 48 h after injection of mercuric chloride in certain epithelial cells with intact cytoplasm (Fig. 2a).

During this period epithelial cells with hypertrophied nucleoli, intensively incorporating the ^{3}H-uridine label, were seen in the undamaged part of the nephron and, less frequently, on the boundary with the zone of necrosis. As a rule peripheral condensed heterochromatin was ill-defined in such nuclei, and the perinucleolar chromatin could not be found. Those nucleoli which contained vacuoles and elements of a nucleolonemal structure were labeled particularly intensively (Fig. 3b).

It follows from these results that disturbance of the structural homeostasis of the renal tissue due to injection of mercuric chloride into the animals was accompanied by inhibition of RNA synthesis in partially damaged cells and also in epithelial cells without any visible ultrastructural changes, located in intact regions of proximal tubules. This reduction in the intensity of ^{3}H-uridine incorporation can be explained to some degree by the inhibitory action of mercuric chloride on enzyme systems [4], and also by the hypoxia developing as a result of a circulatory disturbance, caused by edema of the renal tissue. It can also be postulated that the morphological disturbances arising several hours later in the terminal segment of the proximal tubules, and leading to death and partial necrosis of the epithelial cells, are due largely to the toxic effect of the mercury, whereas the later changes arising in the higher segments are reactive in nature, due to the developing hypoxia. It is reported in the literature that RNA-polymerase activity detected in the ischemic kidney (the renal artery was ligated for 90 min) is considerably depressed, and that synthesis of cytoplasmic protein 2 days after ischemia amounted to only 20% of normal [9].

The intensity of synthesis of rRNA, renewed after inhibition, was much greater than in the epithelial cells of the control animals, and this is evidently an early reparative intracellular regenerative response to the action of the injurious factor.

The fact will be noted that rRNA synthesis, renewed after inhibition, takes place in nuclei in which the transition from peripheral and perinucleolar heterochromatin into the diffuse form takes place, i.e., nuclei in a functionally active state. DNA synthesis may perhaps take place simultaneously in those cells, and subsequently they may proceed into mitosis. For example, double dispersion of chromatin in phytohemagglutinin-stimulated lymphocytes, accompanied initially by stimulation of ^{3}H-uridine incorporation, and later by DNA synthesis, has been described [7]. The presence of epithelial cells in a state of mitosis 48 h after injection of mercuric chloride in the proximal tubules is an indirect indication of this possibility.

The results of this investigation and data obtained previously [1] show conclusively that rRNA synthesis is intensified after damage to the kidneys with mercuric chloride, and the number of cells responsible for DNA replication also is increased. The question of whether these two processes take place simultaneously in the cell remains open. Irrespective of whether the processes of intracellular regeneration and proliferation, i.e., of rRNA synthesis, responsible for de novo ribosome formation, and DNA synthesis, responsible for de novo cell formation, take place simultaneously or successively in an individual cell, these two processes do develop in the renal epithelium during restoration of its structure and function after injury.

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