toration of the mucosa in "acute" atrophy, caused by damage to cells in the S phase, of both hypo- and hyperregenerative types, unlike in "chronic" atrophy, takes place almost identically.

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ELECTRON-MICROSCOPIC AUTORADIOGRAPHIC STUDY OF DNA SYNTHESIS IN TUBULAR EPITHELIAL CELLS OF THE ALBINO RAT KIDNEY WITH NECROTOZING NEPHROSIS DUE TO MERCURIC CHLORIDE

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Since the first study of the effect of mercury salts on animals was published, showing that the site of damage is the epithelium of the renal tubules, this model of necrotizing nephrosis has attracted the close attention of research workers hoping to discover the principles governing restoration of the structure and function of cells of the damaged epithelium. The numerous morphological investigations of the course of destructive and repair processes in the renal epithelium damaged by mercuric chloride so far undertaken at the light-optical and electron-optical levels have demonstrated that destructive changes in the epithelial cells of the proximal renal tubules are heterogeneous in character, and comprise a spectrum ranging from hardly detectable ultrastructural changes and various degrees of partial necrosis to cell death [2-5, 7, 8-11].

The question thus arises: what degree of destructive changes in the epithelial cell of the renal tubule is still compatible with its intracellular regeneration and its ability to reproduce?

To answer this question we used the technique of electron-microscopic autoradiography, whereby the ultrastructure of a damaged cell and certain of its functions, especially its ability to synthesize DNA, can be judged simultaneously.

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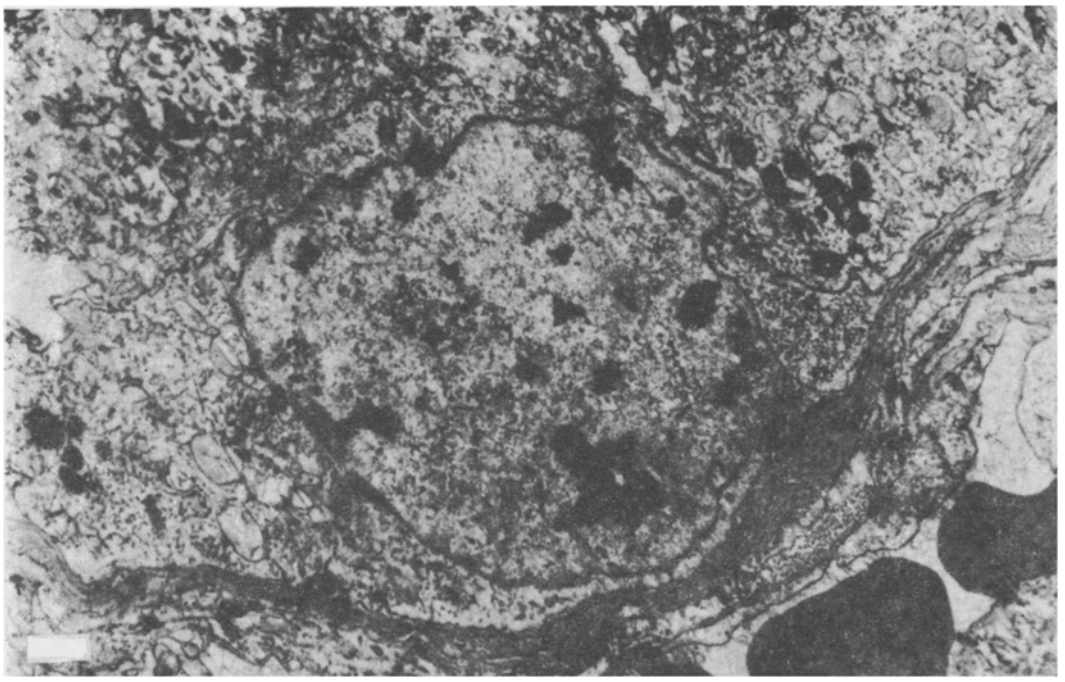

Fig. 1. Ultrastructure of DNA-synthesizing epithelial cell preserved after marked partial necrosis, with a few grains of silver above the nucleus and single mitochondria and lysosomes in the cytoplasm, 72 h after subcutaneous injection of mercuric chloride into rats in a dose of 0.5 mg/0.1 kg (18,000 ×).

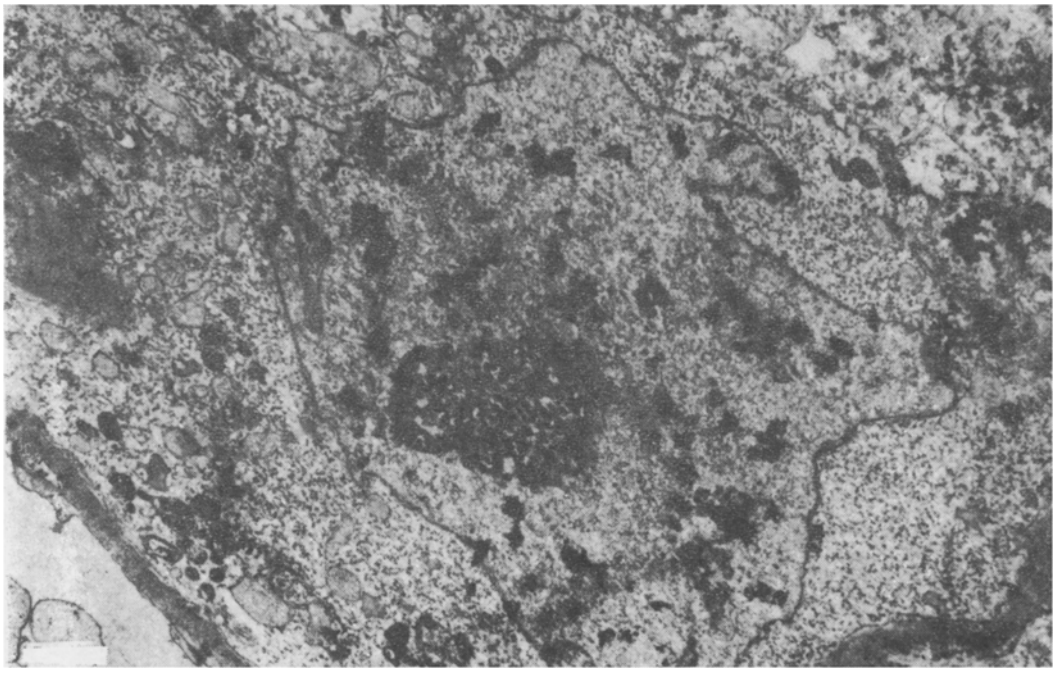

Fig. 2. Ultrastructure of epithelial cell which has lost the apical zone of its cytoplasm, with many ribosomes and polysomes in the residual part of the cytoplasm and intensive concentration of grains of silver above the nucleus, with a large nucleolus, 72 h after subcutaneous injection of mercuric chloride into rats in a dose of 0.5 mg/0.1 kg (21,000 ×).

EXPERIMENTAL METHOD

Mercuric chloride was injected subcutaneously in a dose of 0.5 mg/100 g body weight into noninbred male rats (n = 6) weighing 170-210 g, and this was followed 72 h later by intraperitoneal injection of ^{3}H-thymidine with specific activity of 24 Ci/mmole in a dose of 10 μCi/g.

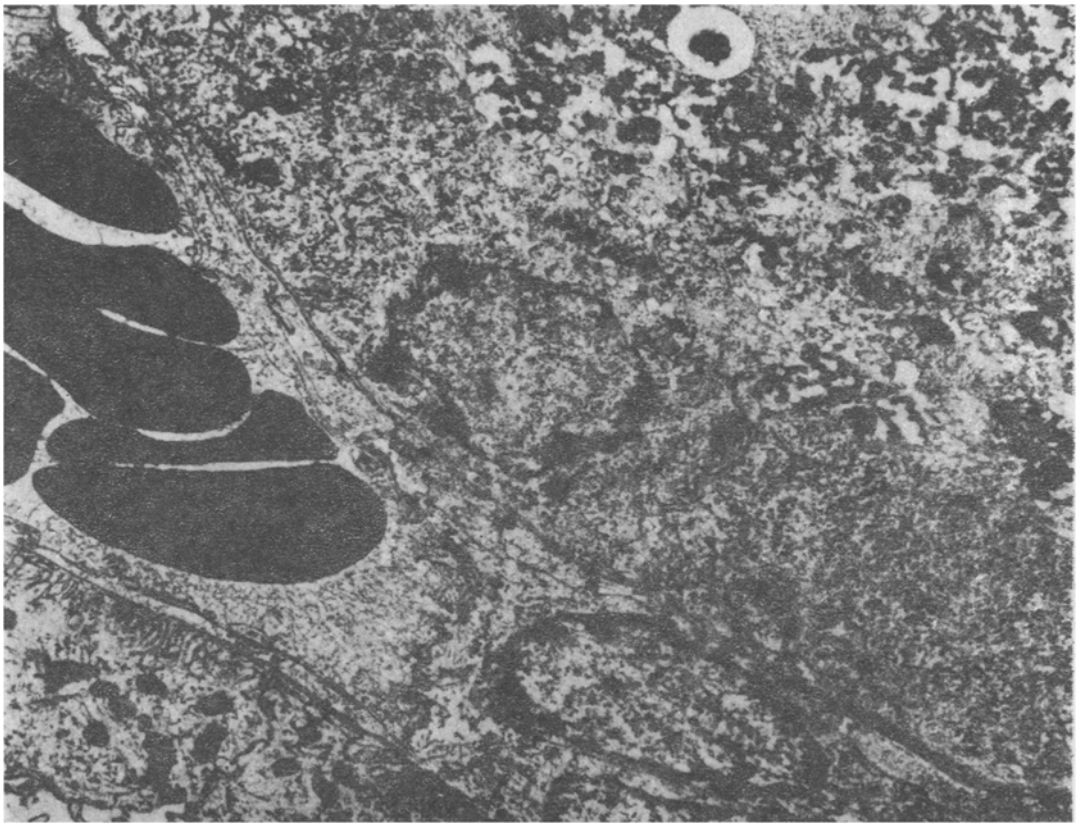

Fig. 3. Damaged epithelial cell at end of synthetic period, with numerous free ribosomes and polysomes and with solitary mitochondria in the cytoplasm 72 h after subcutaneous injection of mercuric chloride into rats in a dose of 0.5 mg/0.1 kg (8000 ×).

Pieces of kidney were taken from the rats killed with ether. 1 h after the injection of H-thymidine and fixed in 1% osmium solution 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. Ultrathin sections 50 nm thick were cut on an LKB (Sweden) ultramicrotome, coated with type M emulsion, exposed for 30 days, developed, and stained with lead citrate and an alcoholic solution of uranyl acetate. The sections were studied under the UEMV-100BR electron microscope.

EXPERIMENTAL RESULTS

Epithelial cells with H-thymidine-labeled nuclei 72 h after injection of mercuric chloride into the animals were found most often in the 2nd and 3rd segments of the proximal convoluted tubules, where the morphological changes were most marked. Incorporation of H-thymidine took place into the nuclei of the epithelial cells, where some of the microvilli were lost together with small areas of cytoplasm, and into nuclei of cells which had undergone marked partial necrosis, with preservation of small borders of cytoplasm around the nuclei.

However, it was noted that the number of grains of silver above the nuclei of the epithelial cells with definite partial necrosis was less than that above the nuclei of cells with only slight damage to their cytoplasm, indicating a weaker intensity of DNA synthesis. Additionally, in tubules with only slight damage to their epithelium, epithelial cells at the end of the synthetic period were found more often than in tubules with marked destruction.

The decrease in the intensity of DNA synthesis in severely damaged epithelial cells can evidently be explained by reduction of the biosynthesizing apparatus and of the mitochondria as a result of the shedding by the cell of part of its cytoplasm, where there was a high concentration of mercury and also by weakening of the activity of enzymes of those mitochondria which remained in the cell, for we know that intracellular repair processes are largely dependent on the level of ATP formation, and in cells damaged by mercuric chloride activity

of succinate dehydrogenase — the key enzyme of the tricarboxylic acid cycle [3, 6], is depressed. The fact that epithelial cells at the end of the synthetic period (in these cells the grains of silver are located around the periphery of the nucleus) are found more often in less damaged tubules can be explained in two ways: either severely damaged epithelial cells begin the S-period later or DNA synthesis in these cells is spread over a longer time. Further investigations are required in order to settle this issue. Data in the literature obtained during the study of mitogen-stimulated human lymphocytes and Chinese hamster ovarian cells indicate that a key role in regulation of the duration of the cell cycle is played by the translation potential of the cell, expressed as the number of ribosomes. A characteristic feature of epithelial cells that have lost a considerable volume of their cytoplasm and are no longer able to synthesize DNA is that their residual cytoplasm contains many free ribosomes and polysomes and only a few mitochondria and lysosomes (Fig. 1).

Because of the absence of serial sections it is impossible to draw a precise conclusion regarding nucleolar morphology in damaged DNA-synthesizing cells, but the presence of large, and sometimes numerous, nucleoli with a well-developed nucleolonema in some cells indicate reactivated RNA synthesis (Fig. 2).

In earlier investigations [1] the authors showed on autoradiographs of semithin sections that the volume of the cytoplasm is increased in damaged DNA-synthesizing cells at the end of the S-period. It is technically difficult to establish under the electron microsope on account of which synthetic processes growth of the cytoplasm of these cells takes place, for we do not know what volume of cytoplasm was preserved in the cell after partial necrosis. However, the discovery of numerous free ribosomes and polysomes and a few mitochondria in the cytoplasm of a few damaged cells at the end of the S-period suggests that growth of the cell from the ending of destructive processes to the end of the synthetic period is mainly due to RNA and protein synthesis and to biogenesis of ribosomes (Fig. 3).

To sum up the results of this investigation it can be said that epithelial cells of the proximal tubules, which have been damaged by mercuric chloride and have undergone marked partial necrosis, can synthesize DNA but, because of the reduced number of organelles in the cell this process takes place more slowly than in only slightly damaged cells. The discovery of numerous free ribosomes in the cytoplasm of damaged DNA-synthesizing cells shows that RNA synthesis and biogenesis of free ribosomes is an earlier regenerative response to the cell to the damaging effect of mercuric chloride. To characterize in greater detail the temporal parameters of intracellular regenerative processes it is therefore necessary to study the dynamics of RNA synthesis by damaged cells and also to study the state of the microtubular cytoskeleton, for there are indications in the literature that many toxins and antimitotic agents destroy the tubules, without which the cell cannot pass through the G_1 period and start DNA synthesis [12].

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