RESTORATION OF THE RENAL TUBULAR EPITHELIUM OF ALBINO RATS IN THE LATE STAGES OF SUBLIMATE NECROTIZING NEPHROSIS

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Mercuric chloride has a nephrotoxic action, and on entering the body it causes necrosis of the tubular epithelium in animals and man, accompanied by acute renal failure of varied degrees of severity [1, 4-6]. Morphological studies have shown that after subcutaneous injection of small doses of sublimate (0.1-0.3 mg/100 g body weight) into animals, the partially damaged epithelium, mainly of the third segment of the proximal portion of the nephron, restores its structural and functional organization very quickly. However, if larger doses of sublimate (0.4-0.6 mg/100 g body weight) are injected into animals, the degree of damage to the epithelium is considerably increased and segments of the proximal part of the nephron at a higher level also are involved in the destructive process. Besides partial necrosis, of a varied degree of severity, large doses of sublimate also cause death of epithelial cells in many nephrons, followed by their desquamation into the lumen of the tubule and denudation of the basement membrane of the tubule over a wide area. The debris thus formed, which consists of a chaotic and dense accumulation of fragmented membranes of microvilli, endoplasmic reticulum, mitochondria, nuclei, and nucleoli, is usually retained in the third segment of the proximal tubule and very rapidly undergoes calcification. The calcified material as a rule delays and distorts regeneration of cells of the tubular epithelium, and also causes obstruction of the urinary tubules.

Since elucidation of the principles governing development of foci of calcification in the kidneys is of both practical and theoretical importance, and since no information shedding light on these mechanisms could be found in the accessible literature, we decided to study the pattern of restoration of the tubular epithelium in the presence of tubular obstruction by debris undergoing calcification.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male rats weighing 170-210 g. The animals were divided into two groups: the rats of group 1 (six animals) served as the control, rats of group 2 (60) received a single subcutaneous injection of mercuric chloride (0.6 mg/100 g body weight), dissolved in physiological saline.

The rats were killed with ether 72 h, 5, 6, 7, 9, 12, 15, 20, and 45 days, and 5 months after the injection of sublimate and pieces of the kidneys removed and fixed in Carnoy's fluid, in 10% neutral formalin solution, and also in a 3% solution of glutaraldehyde in phosphate buffer (pH 7.4), followed by postfixation in a 1% solution of osmium fixative. The tissues were embedded in a mixture of styrene and butyl methacrylate. Semithin sections 0.5-1 μ thick were cut on an ultramicrotome, and stained polychromatically with methylene blue and basic fuchsine. Paraffin sections 8 μ thick were stained with hematoxylin-eosin and for calcium salts by Cossa's method.

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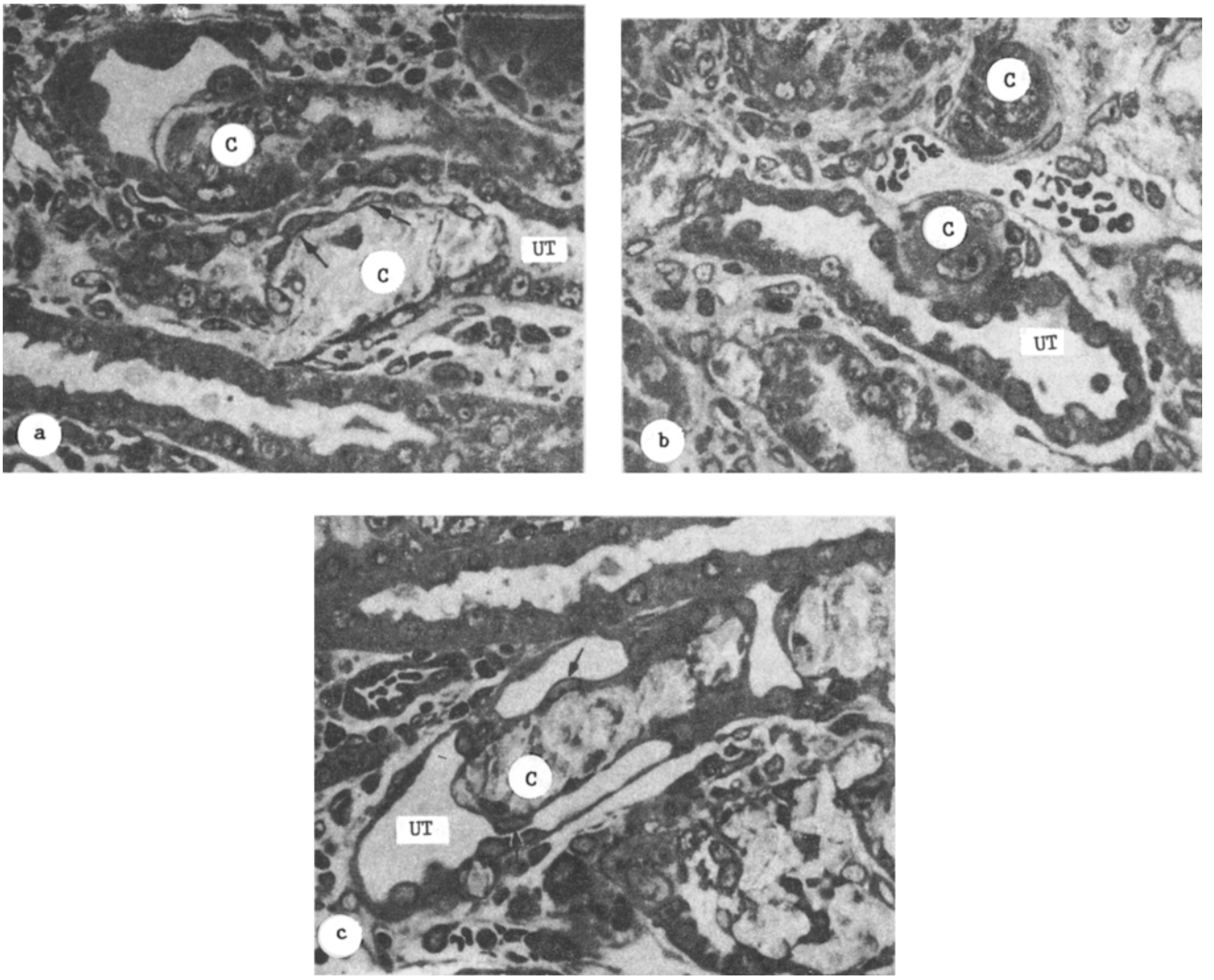

Fig. 1. Outer zone of renal medulla of albino rat 12 days after injection of sublimate in a dose of 0.6 mg/100 g body weight. Magnification: 400. a) Foci of calcification (C) in lumen of urinary tubule (UT) is covered by epithelial cells (arrows). At base of C old basement membrane is destroyed. b) Outside UT, C which has lost its ability to stain with basic fuchsine, is in the stage of resorption. c) Photomicrograph of UT containing a massive C, covered by single epithelial cells (arrows). Cavities formed by stretching with tubular fluid can be seen between the calcified focus and the partially denuded basement membrane.

EXPERIMENTAL RESULTS

The experiments showed that marked calcification of the debris could be observed 72 h after injection of sublimate. In most animals foci of calcification were found most frequently in the outer zone of the medulla, and much less frequently in the cortex. In animals with signs of severe poisoning, and killed with ether, foci of nephrocalcinosis were found equally often in the outer zone of the medulla and in the renal cortex. The absence of calcification in the inner zone of the medulla indicates that the debris was retained in the thick segment of the descending loop of Henle. It was there that massive foci of calcification, obstructing the flow of urine, were found. At sites of accumulation of debris stretching of the urinary tubules was observed, so that the partially damaged epithelial cells which remained assumed a very flattened shape. In the more proximal regions of the nephron, small foci of calcification adherent to the basement membrane at sites of desquamation of the epithelium were more frequently found. During the period of rapid restoration of the tubular epithelium (5th-7th days) the epithelial cells multiplied and covered both the denuded basement membrane and the surface of small calcified foci. Thus small calcified foci were distributed between the basement membrane and the epithelial covering (Fig. 1a).

The adhesiveness of the epithelial cells with calcified debris initially is evidently insufficient, for many desquamated epithelial cells were usually found in these areas of the tubule. Later the epithelial cells forming debris formed a new basement membrane on the side facing the calcified focus; the old membrane, partially or completely deprived of its epithelial cover, was destroyed, and the calcified debris was thus shifted from the lumen of the urinary tubule into the interstitial space. Later the calcified foci lost their ability to stain with basic fuchsine, and became homogeneous and were resorbed. These calcified foci, in different stages of development, were found very often in the kidney 2 weeks after injection of sublimate (Fig. 1b).

Epithelization of large calcified foci took place more slowly because of the considerable denudation of the basement membrane of the urinary tubule. At the same time, in the same preparation, large and medium-sized foci of calcification could be seen outside the tubule and undergoing resorption, whereas massive foci of calcification were still covered with single epithelial cells (Fig. 1c). The morphological pictures are evidence that in the late stages of nephrosis large structures of calcified debris did not present a significant obstacle to the flow of urine, for the increasing pressure in the tubule led to stretching of its walls and to their separation from the calcified focus. The possibility likewise cannot be ruled out that stretching of the walls of the tubule helps to bring about detachment and displacement of fragments of calcified debris, partly or completely covered with epithelium, thus leading to secondary occlusion of the tubule and to a hyperplastic reaction of the epithelium, which many workers have described.

The investigation thus showed that renal tissue is highly capable not only of restoring the tubular epithelium, but also of removing intratubular cell debris by recanalization and by "shifting" it into the interstitial space, where it subsequently undergoes resorption. This is shown by the fact that after 30-45 days, in rats subjected to severe sublimate necrotizing nephrosis, traces of calcified debris were virtually never found in the renal tissue.

However, it must be pointed out that the ability of the renal epithelium to regenerate is not unlimited. This is due to the fact that "denuded" basement membrane is unable to survive for a long time and is destroyed, especially at sites of contact with the debris, thus leading to disappearance of the tubules and their replacement by connective tissue. This was observed particularly often when all three segments of the proximal portion were damaged, as far as the neck of the nephron. There is evidently a certain definite distance which can be covered by tubular epithelium before the denuded basement membrane is destroyed. The retention of debris in the straight segment of the proximal part of the nephron and its rapid calcification are evidently facilitated by the oliguria which develops as a result of a decrease in the velocity of glomerular filtration and leakage of tubular fluid into the interstitial space.

The authors cited in [3], who studied the causes of polyuria, showed that the ultrafiltration coefficient is reduced in rats 24 h after intramuscular injection of sublimate, but the level of glomerular filtration of a single nephron, measured in Bowman's space, was 3 times higher than the glomerular filtration measured in the terminal part of the proximal tubule. They showed by microinjections of ^{3}H-inulin that tubular fluid leaks into the interstitial space.

A study [2] of hemodynamic factors in the pathogenesis of dehydrational, toxic, and postischemic acute renal failure in dogs that, in all these different forms the resistance of the afferent free glomerular arteries is increased, as a result of which the total vascular resistance rises and, in severe cases, simultaneous with spasm of the preglomerular arterioles, there is a compensatory decrease in tone of the efferent vessels. Spasm of the afferent arterioles and dilatation of the efferent vessels lead to reduction of the effective filtration pressure and the velocity of glomerular filtration.

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ELECTRON-AUTORADIOGRAPHIC STUDY OF VIABILITY OF HUMAN HEART CELLS AFTER DEATH

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KEY WORDS: heart; autoradiography; RNA metabolism

The study of functional and morphological changes in the heart cells after death gives a deeper insight into their metabolism and function during life, and also is of direct practical significance for the development of resuscitation and transplant surgery. Determination of the duration of viability of the heart cells after cardiac arrest gives different values depending on the criterion used. A few hours after death the heart beat can be restored [2]. Morphological changes, in the form of severe swelling of the mitochondria, the formation of homogeneous electron-dense residues in their matrix, and fragmentation and vesiculation of elements of the sarcoplasmic reticulum have been observed in rat cardiomyocytes 15-60 min after death [5]. A normal catecholamine content has been found in the hearts of the same animals in the course of 8 h after death [1]. The criteria mentioned above and some others used for the same purpose do not enable the ability of the heart, after arrest, to maintain the normal life of the individual to be reliably confirmed or refuted.

To influence processes taking place in the heart after arrest the first essential is to have information about the basic changes in metabolism in the different cells of the organ. In this paper we give data on a fundamental biological process, namely RNA synthesis, in heart cells at various times after cardiac arrest.

EXPERIMENTAL METHOD

Pieces of atria removed during operations for congenital heart defects in two patients aged 7 and 29 years, and also at autopsy on cadavers of four patients aged 6, 7, 37, and 50 years, the immediate cause of death of whom was combined cardiac and respiratory failure, served as the test material. The time from death to removal of the fragments was 3, 4, 8, and 13 h. During this time the cadavers were kept at room temperature. The excised atrial fragments were cut into pieces measuring 1 mm^3 and were incubated at 37°C for 80 min in medium 199 containing ^{3}H-uridine 100 μCi/ml, with specific activity of 26 Ci/mmole. After incubation the pieces were washed to remove unincorporated ^{3}H-uridine with cold medium 199, fixed in a 2.5% solution of glutaraldehyde and 1% solution of osmium tetroxide, dehydrated, and embedded in a mixture of Epon and Araldite. From each block autoradiographs of semithin sections were prepared ("M" emulsion, exposure 3 days, developer D-19). On the basis of inspection of the autoradiographs of the semithin sections, pyramids

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