ULTRASTRUCTURE OF THE KIDNEYS IN EXPERIMENTAL NEPHROLITHIASIS AND NEPHROCALCINOSIS

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An electron-microscopic study of the kidneys was made in rabbits with experimental oxamide nephrolithiasis and in rats with hypervitaminosis D. The most marked changes were found in the proximal and distal convoluted tubules. A possible role of cytosomes and lysosome-like particles in nephrolithiasis and nephrocalcinosis is postulated. These structures can be regarded as playing an important role in the morphogenesis of human nephrolithiasis.

KEY WORDS: Oxamide nephrolithiasis; hypervitaminosis D; convoluted tubules of the kidney; lysosome-like bodies.

Few electron-microscopic investigations have been made of the kidneys in experimental nephrolithiasis and nephrocalcinosis, and their results are contradictory [4, 10, 11, 14]. Most of the investigations are descriptive in character. Only in a few of them has the attempt been made to study the morphogenesis of the ultrastructures and their role in calculus formation [8, 9]. However, such ideas as have been put forward are purely hypothetical in character, for the etiology and pathogenesis of nephrolithiasis have not been fully explained [1, 2, 3].

The object of this investigation was to study ultrastructures of the nephron with the aim of establishing some general rules governing the morphogenesis of nephrolithiasis.

EXPERIMENTAL METHOD

Experimental nephrolithiasis was obtained in noninbred male rabbits weighing 2500-2800 g by feeding the animals daily with 0.7 g oxamide for 4 months. Nephrocalcinosis was induced in male Wistar rats weighing 120-123 g by intraperitoneal injections of an alcoholic solution of vitamin D_2 in a dose of 50,000 units twice a week for 1.5 months. Pieces of kidney were fixed in cold (4°C) 1% OsO_4 solution in veronal–acetate buffer, pH 7.4, for 1 h. Dehydration was carried out in acetone of increasing concentration. The material was embedded in Epon. Sections 400-500 Å thick were stained with a saturated alcoholic solution of uranyl acetate, counterstained with lead acetate [12], and examined in the IEM-7 and UÉMV-100K electron microscopes.

EXPERIMENTAL RESULTS

The results indicate an early onset of ultrastructural changes under the conditions studied, with metabolic and hemodynamic disturbances for their bases. They differed in degree in experimental nephtolithiasis and nephrocalcinosis and they depended on the duration of the experiment and the dose of the substance given.

In oxamide nephrolithiasis the morphological picture was dominated by an increase in glomerular filtration and also by degenerative and hyperplastic changes in the epithelium of the proximal and distal convoluted tubules. A characteristic feature of the glomeruli was dilatation of the fenestrae of the endothelium,

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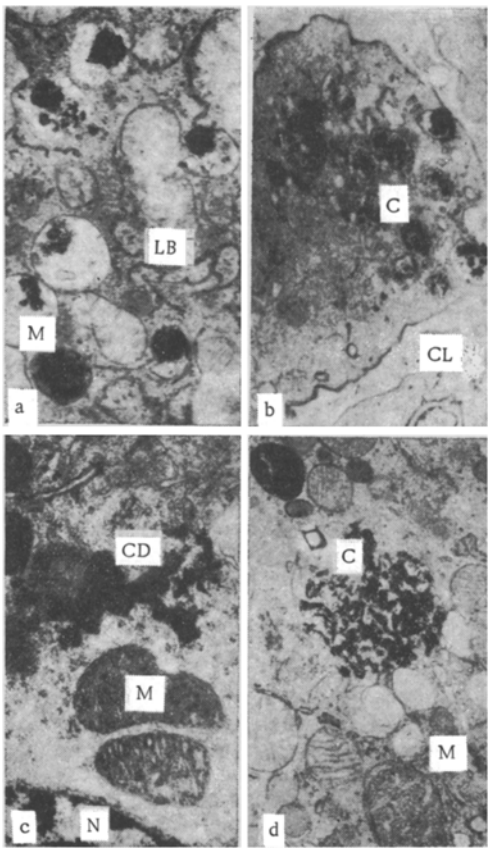

Fig. 1. Cytosomes and lysosome-like bodies in experimental nephrolithiasis (a, b) and nephrocalcinosis (c, d): a) in lumen of capillary (12,640 ×); b) in cytoplasm of epithelium of proximal tubule (16,580 ×); c) calcium deposits in cytoplasm and mitochondrion of distal tubule (25,200 ×); d) cytosomal formation in cytoplasm of proximal tubule (15,500 ×). M) Mitochondria; LB) lysosome-like bodies; C) cytosomes; CL) capillary lumen; CD) calcium deposits; N) nucleus.

the accumulation of vacuoles and micropinocytotic vesicles in its cytoplasm, and an increase in the number of microvesicles of the Golgi complex and of ribosomes of the granular endoplasmic reticulum. In some mitochondria clarification of the matrix, fragmentation of the cristae, and dissociation of their membranes were observed. The basement membrane of the capillaries showed foci of thickening, and fusion of the pedicles was observed on its outer border. The cytoplasm of the podocytes was often translucent and contained lysosome-like bodies, vacuoles, and microvesicles. By contrast with the endothelium, changes in the mitochondria here were more marked and were characterized by a decrease in density of the matrix, by diffuse disorganization of the cristae, and by the formation of myelin-like figures. The accumulation of membrane-like substance in the mesangium and also the appearance of numerous microvesicles and ribosomes in the cytoplasm of the cells must be emphasized.

The results of the study of the epithelial nephron indicated changes in the protein-synthesizing and evacuatory functions of cells mainly of the proximal and distal convoluted tubules. In the proximal segment the microvillous zone of the cells was often destroyed, the basal labyrinth was widened, and the number of vesicles of the paramembranous tubular system was increased. In the apical zones vesicles and vacuoles of the agranular endoplasmic reticulum accumulated. The mitochondria showed destruction of the cristae and myelin-like degeneration, whereas the Golgi complex showed hyperplastic changes and irregular dilatations of the cisterns. Close to the membranes of the granular endoplasmic reticulum the number of ribosomes and polysomes was increased. The appearance of cytosomes and lysosome-like bodies in the cells was noteworthy (Fig. 1a). In the lumen of the capillaries they were found among fragments of cyto-

plasm (Fig. 1b). The impression was obtained that these cell granules participate in the elimination of mineral substances along the lumen of the tubules or the microcirculation. They varied in size, their contents were heterogeneous, and they were bounded by a single membrane. They were composed chiefly of osmiophilic material in the form of large granules and masses located in a very translucent matrix.

Changes in the epithelium of the distal tubules were less marked. Their lumen was empty or contained fragmented microvilli and degenerated mitochondria and cytosomes. Myelin-like transformation of the inner membranes was seen in some mitochondria. The endoplasmic reticulum consisted of vesicles and cisterns with no definite localization and often in contact with the apical vacuoles and microvesicles of the Golgi complex. The cytosomes and lysosome-like bodies resembled those in the proximal segment. However, they were smaller in size and inconstant in location.

The structure of the cells of the thin segment and of the collecting tubules was relatively undisturbed. The distinguishing feature of these portions was the appearance of lipofuscin in the cytoplasm. The cytosomes were situated in the apical and middle parts of the cells. They were seen more often in the capillaries of the juxtamedullary zone and pyramids than in the cortex. Their contents consisted of small vacuoles, individual membranous profiles, and granular material of high electron density.

In the rats with nephrocalcinosis the structure of the filtration membrane of the glomeruli and the cells of the thin segment and the collecting tubules was intact. The most marked changes were found in the proximal and, to some extent, the distal convoluted tubules. They were characterized by the appearance of many polysomes, apical vacuoles, and vesicles, by gross dilatation of the cisterns of Golgi complex and tubules of the endoplasmic reticulum, and by the formation of hernia-like evaginations of the apical cytoplasm. The mitochondria showed focal destruction of the cristae. Sometimes large granules, with increased affinity for osmium, could be seen in their matrix. The appearance of calcium deposits in the cells, which either lay freely in the cytoplasm (Fig. 1c) or were incorporated into cytosomes (Fig. 1d), deserves attention. They could also be found in the basement membrane of the tubule, in the intertubular spaces close to collagen fibrils, and sometimes in the walls of the peritubular capillaries.

The changes were less marked in the distal convoluted tubules. Nevertheless, considerable vacuolation of the cytoplasm and degenerative changes in individual mitochondria were still preserved in this area. Cytosomes were less frequent and the basement membranes did not always contain osmiophilic granules. In the central parts of the cells solitary liposomes could be seen.

These investigations thus did not confirm the view that "ultrafine colloidal particles" participate in calculus formation [13]. In the light of modern views regarding the antagonistic regulation of intracellular functions [7] there are likewise no grounds for suggesting obligate morphological features of nephrolithiasis [15], for the ultrastructures of the cell respond in a fairly stereotyped manner to various types of pathological situations [6] in accordance with the structural and functional specialization of the nephron [5].

During calculus formation, as the experiments described above show, an important role is played by lysosome-like bodies and cytosomes, which eliminate mineral substances from the cell. Disturbance of the evacuatory mechanisms leads to intra- and extracellular calcium deposition and the development of nephrocalcinosis, which is not an essential condition for nephrolithiasis. Similar relations evidently apply also to human nephrolithiasis.

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