ULTRASTRUCTURAL CHANGES IN THE NEPHRON DURING EXPERIMENTAL ISCHEMIA AND POSTMORTEM SURVIVAL

T. M. Povalii

UDC 616.61-005.4-092.9-091.8

Ultrastructural analysis showed differences in the response of the epithelium of the proximal and distal portions of the nephron during postmortem survival of the kidney and its experimental ischemia in noninbred albino rats. During autolysis of the organ in the cadaver intracellular edema develops in the tubular epithelium and characteristic changes appear in the ultrastructure of the mitochondria. Under the conditions of experimental ischemia, marked changes in the lysosomal system of the cells, swelling of the mitochondria, and considerable dilatation of the cisterns of the endoplasmic reticulum and the Golgi lamellar complex take place against the background of intracellular edema.

Key words: autolysis; postmortem survival of organs; ischemia; edema.

A leading place in medical and biological research into the problem of organ and tissue transplantation is occupied by the study of cell viability [2]. This problem has become particularly acute in connection with the use of cadaveric organs for transplantation, and this is especially true of the kidney, one of the organs most frequently transplanted [1, 3]. An important condition for normal functioning of an organ after transplantation is a short stay in the cadaver after death. The effect of this "survival" on the structure and function of the transplanted organ has frequently been studied by the use of experimental ischemia in the living organism as the model [6, 7, 9, 11, 15], whereas the donor's kidney is taken as a rule a short time after death of the organism. On the other hand, the study of the biochemical and morphological characteristics of cells during autolysis as a rule is carried out on isolated organs under artificial conditions [4, 5, 8-10].

The object of this investigation was to study ultrastructural changes in the cells of the proximal and distal portions of the nephron of noninbred albino rats during experimental ischemia of the kidney and during postmortem survival of the organ in the cadaver.

EXPERIMENTAL METHOD

Experiments were carried out on male rats divided into two groups with 20 animals in each group. Under pentobarbital anesthesia a silk ligature was passed around the left renal artery of the animals of group 1 and tied for 15, 30, 60, and 120 min, after which material was taken from the ischemia kidney. The effect of ischemia for each of these periods on the ultrastructure of the renal epithelium was studied in five animals.

The animals of group 2 were decapitated. Material was taken from the left kidney 15, 30, 60, and 120 min after death of the animal. In this case also five rats were studied at each time. The material was treated by the usual methods for electron microscopy. Specimens were examined in the Hitachi IIE electron microscope.

Laboratory of Electron Microscopy, Central Research Laboratory, N. I. Pirogov Second Moscow Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 79, No. 3, pp. 118-121, March, 1975. Original article submitted August 6, 1974.

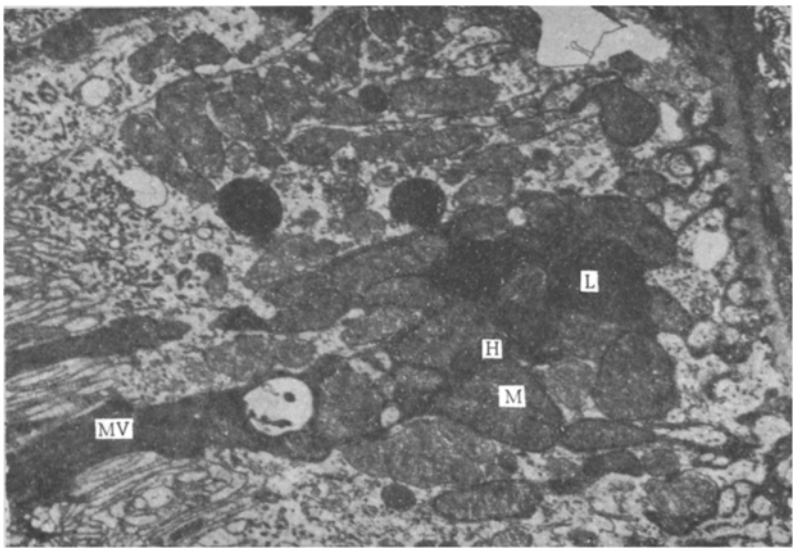

Fig. 1. Ultrastructure of cell of proximal portion of nephron during postmortem survival of the kidney in the cadaver for 15 min (9600×): H) electron-dense hyaloplasm, L) lysosome, M) mitochondrion, MV) microvilli of brush border.

EXPERIMENTAL RESULTS

During postmortem survival of the kidney in the cadaver the most characteristic sign of injury of the tubular epithelium was intracellular edema accompanied by reorganization of the shape of the epithelial cells and by ultrastructural changes in them. The configuration of the epithelial cell surface in the proximal portion of the nephron 15 min after death of the animals was appreciably altered as a result of widening of the basal folds and a reduction in their depth and also through thickening and shortening of the microvilli of the brush border. The cytoplasmic matrix of some cells was "watery" and pale. In some cells areas of hyaloplasm of high electron density were found. The microvilli in these areas appeared swollen and confluent. The appearance of mitochondria with an electron-dense matrix and a shorter distance between the cristae in the cells of the proximal portion was particularly noticeable (Fig. 1).

The changes described above were joined 60 min after the animal's death by responses of the lamellar complex of Golgi, in which the cisterns were widened and fragmented. With a further increase in the period of postmortem survival of the kidney the intensity of these ultrastructural changes in the epithelium of the proximal portion of the nephron increased.

The ultramicroscopic responses of cells in the distal portion of the nephron of the "surviving" kidney were similar to those described above in the epithelium of the proximal portion. However, the characteristic response of the epithelium in the distal part of the nephron was a change in its biosynthetic structures, in the form of sharp dilatation of the cisterns of the endoplasmic reticulum and fragmentation and dilatation of the cisterns of the Golgi lamellar complex, appearing 15 min after the animal's death. The degree of these changes grows sharply at later periods of postmortem survival.

It can thus be concluded from these observations that during autolysis the tubular epithelium loses the specific differentiation of the apical and basal cell surfaces and is characterized by morphological changes in the energy-producing and biosynthetic systems. These lesions are evidently based on structural and functional disturbances of permeability of the cell membranes.

The morphological picture of the damage under ischemic conditions was rather different.

The most characteristic features of ischemia associated with intracellular edema were changes in the lysosomal apparatus of the cells, especially marked in the epithelium of the proximal portion, and also swelling of the mitochondria and sharp dilatation of the cisterns of the endoplasmic reticulum and Golgi lamellar complex, especially in cells of the distal portion of the nephron.

After ischemia for 15 min an increase in the number of lysosomes and phagosomes and an increase in their polymorphism were observed in the cells of the proximal portion. The characteristic picture after short periods of ischemia (up to 60 min) was the fusion of two lysosomes and also close contact between ly-

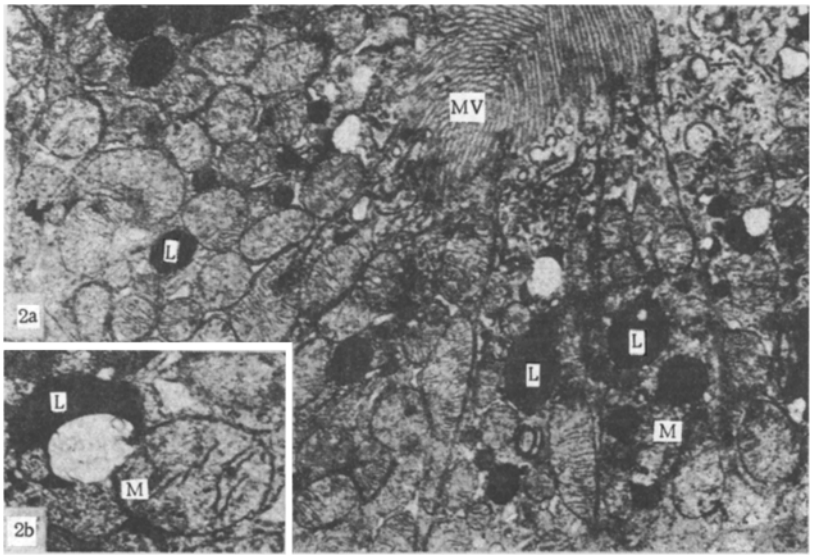

Fig. 2. Ultrastructure of a cell of the proximal part of the nephron after experimental ischemia lasting 15-30 min (27,000×): a) general view of section; b) place of contact of lysosome with mitochondrion. Legend as in Fig. 1, 11,200×.

sosomes and mitochondria (Fig. 2). In the zone of contact the outer membranes of the mitochondria were damaged (Fig. 2). An increase in the number of swollen mitochondria, as shown by translucency of their matrix and disorganization of their cristae, also was observed.

After ischemia for 60-120 min the lysosomal apparatus of the cells of the proximal portion became morphologically less active. The polymorphism of the lysosomes was less marked, the number of contacts of lysosomes with each other and with the remaining cell organelles was reduced. After ischemia for 2 h, against the background of marked intracellular edema of the endothelium of the proximal portion, sequestration of individual areas of endothelial cells was found. After this period of ischemia the mitochondria appeared swollen and fragmented. Most of the lysosomes were circular, of average size, and with homogeneous contents. Phagosomes were rare. Cisterns of the Golgi lamellar complex were sharply dilated and fragmented.

In the distal portion of the nephron ischemia for 15 min induced marked vacuolation of the cytoplasm of the endothelium and swelling of the mitochondria. However, the sharp changes in the lysosomal apparatus occurring in the endothelium of the proximal part of the nephron were not found in cells of the distal portion. With an increase in the duration of ischemia, the rapid development of intracellular edema was characteristic of cells of the distal portion. Most epithelial cells of the distal portion 120 min after application of the ligature had a smooth surface, for the depth of the basal folds was considerably reduced and, in some parts of the cell, they had disappeared completely. The cytoplasm of the cells was of low electron density and rich in vacuoles of different sizes. The mitochondria were mostly swollen, with solitary cristae. The Golgi lamellar complex, composed of separate vesicles and vacuoles, was scattered throughout the cytoplasm.

It can be concluded from the analysis of these observations that intracellular edema took place during postmortem survival of the kidney and ischemia, as a result of disturbances of the microcirculation in the organ. However, postischemic changes in the ultrastructure of the lysosomal, energy-producing, and protein-synthetic systems of the epithelial cells are difficult to distinguish from changes in the cells during postmortem survival of the organ. In addition, the dynamics of development of intracellular edema differs in these two conditions, on the one hand, and in each part of the nephron of the kidney under the conditions of autolysis and ischemia, on the other hand. Consequently, there is a marked difference in the cellular metabolism of the epithelium of the nephron during ischemia and postmortem survival. The use of a model of experimental ischemia to study the viability of a transplanted organ thus cannot give a complete picture of the state of the working cells of the organ after its survival in the cadaver.

LITERATURE CITED

1. I. D. Kirpatovskii and N. A. Bykova, Transplantation of the Kidney [in Russian], Moscow (1969).
2. Yu. M. Lopukhin, B. M. Cheknev, E. G. Shifrin, et al., in: Current Problems of the Transplantation of Organs and Tissues [in Russian], Moscow (1969), p. 146.
3. B. V. Petrovskii, G. M. Solov'ev, V. I. Govallo, et al., Transplantation of the Kidney [in Russian], Moscow–Warsaw (1969).
4. M. L. Cook, L. Osvaldo, J. Jackson, et al., Lab. Invest., 14, 623 (1965).
5. J. L. Ericsson and P. Biberfeld, Acta Path. Microbiol. Scand., 70, 215 (1967).
6. L. Harrison, J. Surg. Res., 12, 765 (1972).
7. R. Keeber, Canad. J. Physiol. Pharmacol., 46, 739 (1968).
8. H. Latta, L. Osvaldo, J. D. Jackson, et al., Lab. Invest., 14, 635 (1965).
9. L. Osvaldo, J. D. Jackson, M. L. Cook, et al., Lab. Invest., 14, 603 (1965).
10. R. Reimer and C. Gonote, Lab. Invest., 26, 347 (1972).
11. R. Reimer and D. D. Jennings, Lab. Invest., 25, 176 (1971).
12. R. Reimer and D. D. Jennings, Lab. Invest., 25, 253 (1971).
13. W. Thoems, Mikromorphologie der Nephron nach temporärer Ischemie, Berlin (1964).
14. J. Wellington and R. Conrad, Acta Path. Microbiol. Scand., 80, Suppl. 233, 217 (1972).
15. M. T. Vogt and E. Farber, Am. J. Path., 53, 1 (1968).