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Close correlation is known to exist between the form of deposition of products of enzyme histochemical reactions involving the use of tetrazolium salts, ultrastructural changes in the mitochondria, and the functional capacity of the muscle cells of the isolated heart [1]. On this basis types of deposition of the enzyme histochemical reaction product reflecting the degree of degenerative and necrobiotic changes in a preserved organ have been defined. A diffuse-granular type of reaction product corresponds to the normal average physiological state of the cell. Intensification of the diffuse component in this type of reaction is a histochemical sign of functional (relating to energy) stress on the cell. The granular and dispersed types of diformazan precipitation are connected with profound but reversible disturbances in the energy metabolism of the mitochondria. Finally, the secondary diffuse type of deposition of enzyme histochemical reaction products corresponds to profound degenerative and autolytic changes in the parenchymatous cells of the preserved organ. These criteria were used by the writers to compare the morphological and functional state of the kidneys preserved by a nonperfusion method in various solutions.

EXPERIMENTAL METHOD

Kidneys of mongrel dogs weighing 10-12 kg were preserved. The preserving solutions consisted of 0.9% NaCl solutions, Collins' solution [6], and VNIKIÉKh solution [2]. The last two solutions are equivalent in Na and K concentration to intracellular fluid, but in their anionic composition they are mixtures of inorganic and nonmetabolized ions. Phosphate anions predominate in Collins' solution and sulfate ions in VNIKIÉKh solution. Solutions ITiIO No. 1 and ITiIO No. 2, developed in the writers' own Institute, also were used. In ITiIO No. 1 solution, by contrast with VNIKIÉKh solution, the anions are organic acid residues (aspartate, succinate, acetate), which are natural metabolites. Solution ITiIO No. 2, like Collins' solution, is based on phosphate buffer with the addition of succinate, citrate, and adenine. The vessels of the isolated kidneys were flushed out through the artery under a pressure of 70-80 mm Hg with the solutions cooled to 2-4°C, and were then stored in the same solutions at a temperature of 0-2°C. Kidneys were subjected to heat ischemia (20°C). The time of storage of the kidneys in the preserving solutions and the duration of heat ischemia were 24 h. Material for general histological study was fixed in 10% neutral formalin and embedded in paraffin wax; sections were stained with hematoxylin and eosin. For the enzyme histochemical reactions cryostat sections were cut from unfixed material to a thickness of 10 μ . Succinate dehydrogenase (SDH) activity was demonstrated in the sections [4]. Material for electron-microscopic study was treated in the usual way, after which ultrathin sections were examined in the IEM-100B microscope.

EXPERIMENTAL RESULTS

The most severe necrobiotic changes in the tubular epithelium, especially marked in the proximal portion of the nephron, were found in kidneys exposed to heat ischemia. Correspondingly, histochemical investigation revealed dispersed and secondary diffuse types of precipitation of the reaction products in the cytoplasm of the epithelium of the proximal tubules and collecting tubules. Meanwhile a sharp decline was found in SDH activity in the proximal tubules and also in cells of the renal glomeruli. Similar histological and histochemical changes also were found in kidneys preserved in 0.9% NaCl solution. However, SDH activity in

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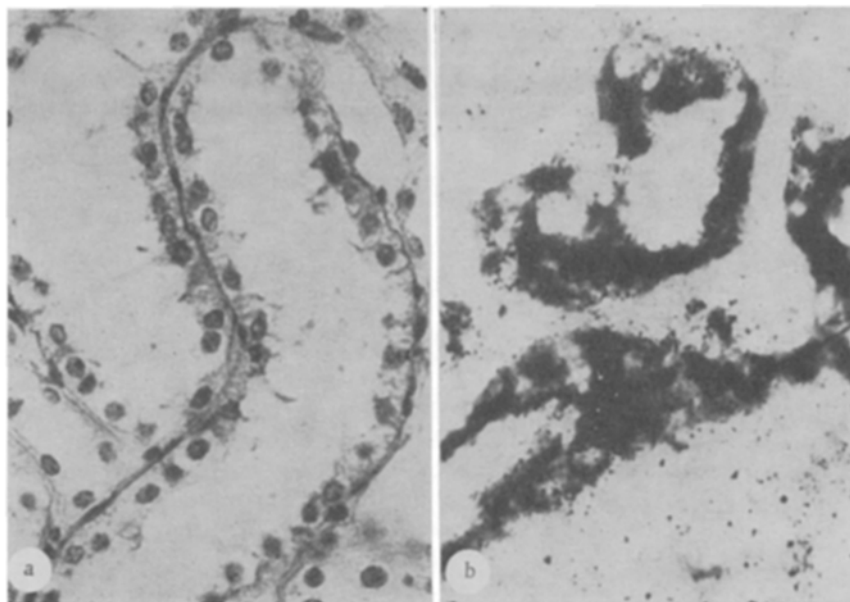


Fig. 1. Preservation of the kidney in ITiIO No. 1 solution for 24 h; a) destructive changes in collecting tubules (hematoxylin and eosin, 800 \times); b) conglomerates of reaction products for SDH in epithelium of collecting tubules (Nachlas' reaction with nitro-BT; 800 \times).

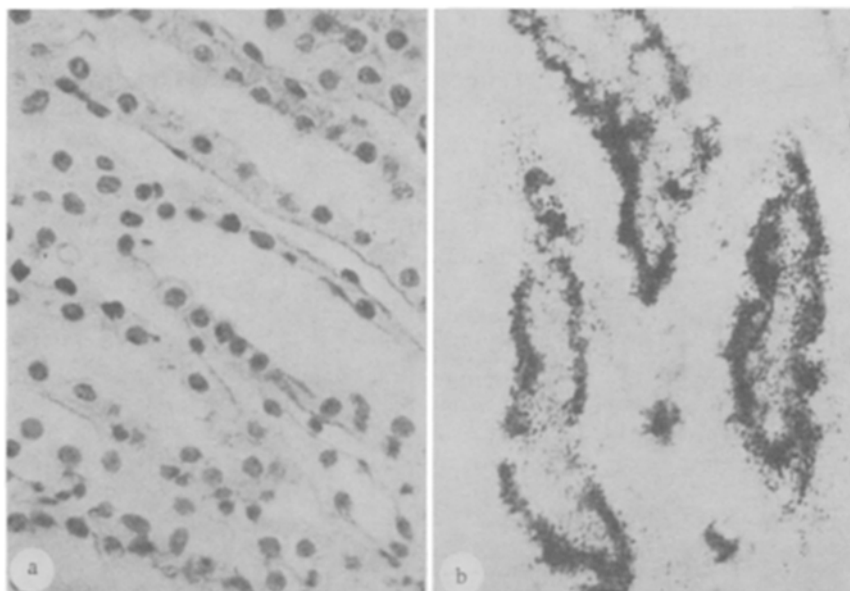


Fig. 2. Preservation of the kidney in ITiIO No. 2 solution for 24 h: a) negligible changes in structure of collecting tubules (hematoxylin-eosin, 800 \times); b) dispersed, finely granular type of deposition of diformazan granules in epithelium of collecting tubules (Nachlas' reaction for SDH; 800 \times).

the proximal tubules and cells of the capillary glomeruli was considerably higher than in the kidney exposed to heat ischemia.

After preservation in ITiIO No. 1 solution less severe histological and histochemical changes were found than after preservation in 0.9% NaCl solution. The glomeruli and proximal tubules appeared better preserved. A combination of the dispersed type of deposition of diformazan granules in the cells and in the tubular epithelium with the usual ultramicroscopic

structure of the podocytes and the slight degree of vacuolation of the mitochondria in the epithelium of the proximal portion of the nephron reflected the relatively high degree of preservation of the filtration and reabsorption regions of the nephron. At the same time, signs of destructive changes could be detected in the collecting tubules (Fig. 1a). Large conglomerates of enzyme histochemical reaction products were observed in the cytoplasm of their epithelial cells (Fig. 1b), evidence of considerable changes in the mitochondria. This was confirmed by an electron-microscopic investigation. The dispersed type of deposition of diformazan was discovered in the tubular epithelium of the loop of Henle, indirect evidence of reversible disturbances of the mitochondria.

After preservation in VNIKIÉKh solution satisfactory integrity of the histological structure of the nephrons was observed. SDH activity was high in the cytoplasm of the tubular epithelium. The dispersed type of precipitation of diformazan granules of different sizes predominated. Only in a few areas of collecting tubules could histochemical signs of damage to structures of the epithelial cells be noted, in the form of intensive and diffuse staining of their cytoplasm and deposition of large diformazan droplets.

The highest degree of morphological integrity of the structural elements of the kidney at the cellular (Fig. 2a) and subcellular levels and of SDH activity were observed after preservation in ITiO No. 2 solution. Only the dispersed, finely granular type of deposition of diformazan granules was observed in the cytoplasm of the epithelial cells of all parts of the nephron and also in the collecting tubules (Fig. 2b). The ultrastructure of the mitochondria in the epithelia of all parts of the renal tubules was indistinguishable from normal. Only in the cells of the proximal portion were membranes of the mitochondrial cristae a little shortened, and their matrix edematous.

The results of these morphological observations corresponded fully to those of functional investigations which confirmed the best preservation of isolated kidneys after keeping for 24 h in ITiO No. 2 solution.

The experiments thus showed that the type of deposition of diformazan precipitated during enzyme histochemical reactions for SDH reflects sufficiently accurately structural and functional changes in the parenchymatous cells of isolated kidneys. Considering the results of the writers' previous histochemical and morphological investigations of the isolated heart, it can be concluded that the above-mentioned types of deposition of tetrazolium reaction products can be used to judge the degree of integrity of organs and tissues preserved by different methods.

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