ELECTRON-MICROSCOPIC INVESTIGATION OF THE RESIDUAL KIDNEY AFTER UNILATERAL NEPHRECTOMY AND COMBINED IMMUNOSUPPRESSIVE TREATMENT

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The effect of combined immunosuppressive treatment (Imuran, prednisolone, local x-ray irradiation) on the dog kidney for 5 months was studied by the electron microscope. Administration of immunosuppressive agents had a cytotoxic action and produced moderately severe lesions in all parts of the nephron. On the basis of the results it is recommended that combined immunosuppressive measures be used with more care and that other methods of treatment not producing serious morphological changes in the kidneys be sought.

Few morphological studies of the action of immunosuppressive agents on the kidney tissues have been made, and they have mainly used histological methods. Sheil et al. [23] injected azathioprine and azaserine into dogs and found no histological changes in autografts of the kidneys. Vriesman et al. [25] studied various enzymes in autografts of the kidneys of dogs receiving azathioprine and actinomycin C. They concluded that this treatment for 70 days caused no morphological changes in the kidneys. Other workers emphasize that during immunosuppressive therapy more severe lesions are found in the blood vessels of allografted kidneys [1, 17, 21, 22], while in the late stages of the experiment and after clinical operations sclerotic changes develop in the blood vessels and stroma [2, 8, 13, 18]. However, it is difficult to differentiate these changes from those resulting from immunological conflict.

The object of the present investigation was to study the effect of combined immunosuppressive therapy on the ultrastructure of the residual kidney after unilateral nephrectomy. No such investigations could be found in the literature.

EXPERIMENTAL METHOD

The material studied comprised 21 incision biopsy specimens of the kidneys from 8 dogs. Combined treatment was given to 3 experimental animals from which one kidney and the spleen were removed. The kidneys of 3 healthy dogs formed the first control, and biopsy material from the kidneys of 2 untreated dogs from which the contralateral kidney was first removed formed the second control. During the 3 days before the operation the experimental animals received Imuran (azathioprine) in a dose of 3 mg/kg, the same dose was given for 3 days after the operation, and from the 4th day the dose was reduced to 2 mg/kg daily until the end of the experiment. On the day of the operation and until the end of the experiment prednisolone was given in a daily dose of 2-5 mg/kg. During the first 2 weeks local x-ray irradiation of the kidney was carried out in a dose of 100-150 R on alternate days. The usual analyses of the blood and urine served to check the state of the kidney function. At the moment of unilateral nephrectomy and 7 and

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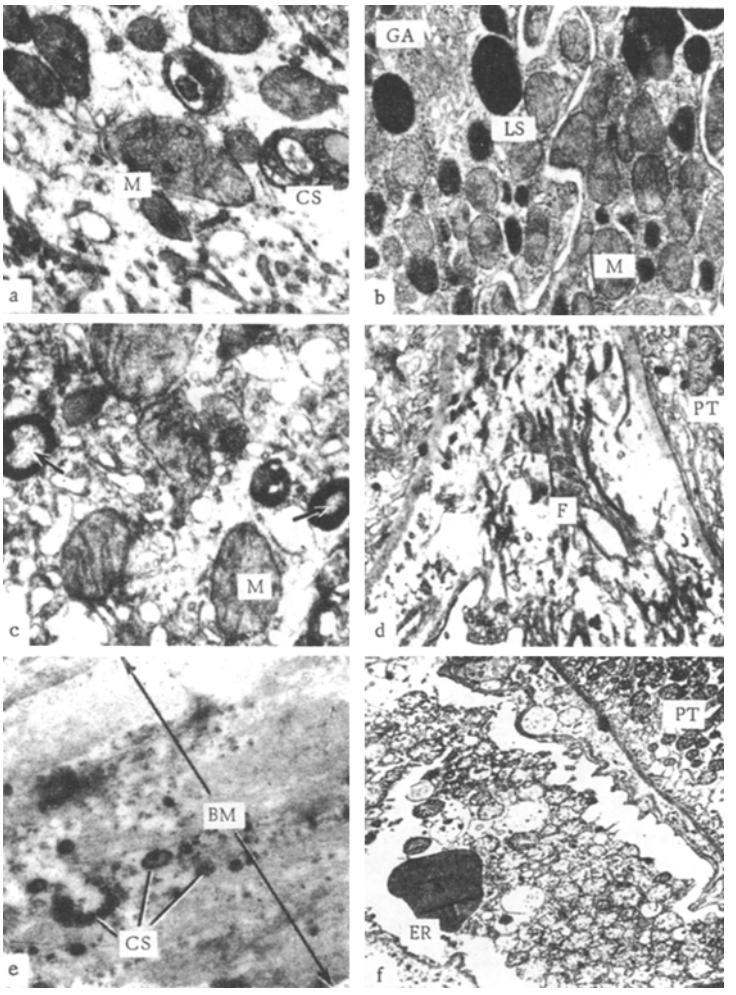

Fig. 1. Changes in ultrastructure of the kidney during immunosuppressive treatment: a) proximal tubule. Swelling of mitochondria (M) with simplified structure of cristae. CS) cytosome. 18,000 ×; b) cytoplasm of epithelium of proximal tubule contains many lysosomes (LS) and a well-marked Golgi apparatus (GA), 12,000 ×; c) myelination figures are visible in cytoplasm of epithelium of proximal tubule (indicated by arrows), 20,000 ×; d) fibrin fibers (F) in interstitial tissue. PT) proximal tubule, 12,000 ×; e) thickening of basement membrane (BM) of proximal tubule and deposition of calcium salts (CS), 30,000 ×; f) conglomeration of fragmented mitochondria in lumen of efferent capillary. ER) erythrocyte, 6000 ×.

21 days and $1\frac{1}{2}$ months after the beginning of treatment incision biopsy specimens were taken; the dogs were sacrificed after $3\frac{1}{2}$ and 5 months. Tissue from the renal cortex was fixed by Caulfield's method and embedded in methacrylates and Araldite. Sections were examined in the UÉMV-100B electron microscope.

EXPERIMENTAL RESULTS

The electron-microscopic study of kidney tissue taken at operation after 3 days of treatment with Imuran showed that the structure of most nephrons was indistinguishable from that of the nephrons of the first control group of animals. However, there were some proximal convoluted tubules in which the villi of the brush border were detached, thinner than normal, and showing signs of lysis. The mitochondria in these tubules varied in shape and size. Some of them contained vacuoles and in others the cristae were

fewer in number and less complex in structure (Fig. 1a). The cytoplasm contained rather more cytosomes than in the first control. Cases are described in the literature in which degenerative changes were found in the epithelium of the renal tubules after administration of Imuran alone [16].

Seven days after the operation and treatment foci of splitting of the pedicles of the pericytes on the capillary membranes were found. In the proximal and distal tubules, besides the changes in the epithelium described above there was a well-defined focal thickening and loosening of the structure of the basement membranes with inclusion of electron-dense masses (calcium salts). Besides the moderately severe degenerative changes described above, intracellular hyperplasia was apparent. A marked increase in the number of mitochondria (3-4 times more numerous than in the first control) and of the membranes and vacuoles of the Golgi apparatus and free and fixed ribosomes, widening of the cisterns of the rough and agranular reticulum, and some increase in density and an even distribution of the chromatin granules in the nuclei were observed in the epithelium of the proximal and distal tubules and the collecting ducts. Many lysosomes of different sizes and differing in the density of their contents were found in the cytoplasm of the epithelium mainly of the proximal tubules (Fig. 1b). The number of ribosomes was also sharply increased in the endothelium of the interstitial capillaries. In the kidneys of the second control group of animals at these times no degenerative changes were found, the intracellular hyperplastic changes were not so pronounced, and the number of lysosomes was much smaller.

Development of hyperplasia was demonstrated electron-microscopically by Anderson [15] as early as 96 h after unilateral nephrectomy, while Zufarov and Gontmakher [7] observed similar changes 15 days after the operation. Threlfall et al. [24] found an increase in the size of the residual kidney and an increase in its RNA content by 35-40%, and an increase in its DNA content by 25%, 14 days after unilateral nephrectomy. It must be emphasized that in the present experiments hyperplasia of the intracellular structures in the residual kidney was probably due not only to natural compensation, but also to some extent to repair in response to the harmful action of the immunosuppressive treatment.

The outer membranes of some mitochondria in the epithelium of the proximal and distal tubules became dense and were converted into myelin-like figures 21 days and $1\frac{1}{2}$ months after the operation (Fig. 1c). Many cytosomes in which fragments of disintegrated mitochondria or myelin-like structures, fragments of membranes, and lipids were visible, were discovered. The basement membranes of the tubules were often thickened, and extensive areas imbibed with fibrin were found in the interstitial tissues (Fig. 1d). Meanwhile hyperplasia in the epithelial cells of the nephron was clearly defined. However, the number of free ribosomes and, in particular, of lysosomes was reduced $1\frac{1}{2}$ months after treatment and was almost indistinguishable from their number in the second control group of animals. Relative stabilization of metabolism with an increase in the number of ribosomes and lysosomes to the "normal" level was evidently associated with the discontinuing of x-ray irradiation at this period.

By $3\frac{1}{2}$ and 5 months after the operation the ultrastructure of most neurons was still relatively intact. Meanwhile in the epithelial cells of the tubules degenerative changes were found in the mitochondria and brush border. Thickening of the basement membranes of the tubules was more common and was now mixed focal and diffuse in character, often with inclusions of electron-dense masses (Fig. 1e). Fragments of membranes and conglomerations of mitochondria were found in the lumen of the efferent capillaries (Fig. 1f). In the interstitial tissues along the course of the basement membranes of the tubules there was an increase in the number of collagen fibers compared with the first control group of animals, but no difference could be found in the degree of collagen formation compared with the second control group of animals.

This electron-microscopic study thus showed that local x-ray irradiation of the kidney and the immunodepressive measures taken exerted a cytotoxic action and caused damage to all parts of the nephron. The lesions were focal in character, so that the kidney as a whole remained relatively intact. This conclusion is confirmed by the results of function tests (blood nonprotein nitrogen and creatinine, 24-hourly diuresis), which were normal at all stages of the experiment.

The most distinct lesions were observed in the proximal convoluted tubules, presumably because of the morphological and functional characteristics of this part of the nephron [3]. The frequently observed fact that in whole-body irradiation as well as after local irradiation of the kidney region the proximal tubules of the kidney are affected first, and this is followed by the development of nephrosclerosis [2, 4, 9, 11, 20], must also be remembered. Sclerosis may also be stimulated by prednisolone, whose fibroplastic qualities are well known [10, 12]. However, no differences could be found in the degree of collagen

formation in the interstitial tissues in the experimental and control groups of animals. This process must evidently be regarded as an adaptive response aimed at strengthening the connective tissue skeleton of the residual kidney.

During the experiment the structural and functional state of the nephron apparently passed through two stages, as reflected demonstratively by the pattern of changes in the ribosomes and lysosomes. Before $1\frac{1}{2}$ months there was an increase in the number of ribosomes and lysosomes, especially in the first week after immunodepressive treatment. The number of ribosomes and lysosomes then fell. The marked activation of synthesis in the first stage, when its intensity was higher than in the second control group of animals, was certainly due to the action of the immunosuppressive agents and, in particular, to x-ray irradiation. After discontinuation of the x-ray irradiation, relative stabilization of metabolism was observed. By the end of the experiment the degenerative changes in the epithelium and, in particular, in the basement membranes of the tubules were slightly increased in intensity, an unfavorable sign indicating depression of the synthetic power of the cells and the predominance of degenerative changes over repair. There are many examples in the literature to show that the course of pathological processes in response to a wide variety of factors takes place in a series of phases [5, 14, 19]. The cytotoxic effects of the immunosuppressive agents used obtained in these experiments lead to the conclusion that more care must be exercised with their combined administration and that other methods of treatment free from the risk of serious morphological changes in the kidneys must be sought.

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