INJURY TO AND REGENERATION OF THE RENAL EPITHELIUM AFTER CRUSHING OF THE SOFT TISSUES OF THE LIMBS

A. N. Gansburgskii

UDC 617.57/.58-001.35-092.9-07:616.61-018.73-003.93

After crushing of the soft tissues of the limbs in rabbits the dynamics of the cell composition in the proximal thin and thick regions of the loop and the distal portions and collecting tubules of the kidneys was studied. The area of the nuclei in the epithelium of the proximal and distal tubules and of the collecting tubules was determined planimetrically. Regeneration of the epithelium of the nephrons and collecting tubules was found to correlate with the degree of injury, increased mitotic activity was associated with an increase in the relative number of pathological mitoses, and the mean area of the cell nuclei was increased.

KEY WORDS: crushing of soft tissues; regeneration of renal epithelium; mitotic activity; dimensions of nuclei.

Most information on regeneration of the kidneys has been obtained by investigations of processes after resection or nephrectomy. However, in clinical practice it is more frequent to find diffuse degenerative and necrotic changes in the parenchyma associated with circulatory disturbances or the action of toxic agents [5, 7, 8]. During crushing of the soft tissues of the limbs, a combination of these factors exists in the kidneys [3, 4]. However, the dynamics of these changes and, in particular, of repair processes is hardly reflected at all in the literature.

The investigation described below was carried out to study these dynamics.

EXPERIMENTAL METHOD

The soft tissues of both hind limbs of 50 sexually mature rabbits were crushed for 1.5 h by means of special clamps corresponding in size to the thigh [3]. Seven of the rabbits died between 9.5 h and 17 days after the experiment; the rest were decapitated between 9 and 9.30 a.m. from 4.5 to 24 h and from 2 to 50 days later (at least two animals at each time). The kidneys of three rabbits served as the control. The material was treated by general histological and histochemical methods. The areas of projection of the nuclei

TABLE 1. Mitotic Index (in %) of Epithelium of Nephrons and Collecting Tubules in Control and after Crushing of Soft Tissues (M ± m)

Region of nephron or collecting tubules	Control	3rd day	9th day	15th day
Proximal	0,095±0,048	4,2±0,373	14,833±0,698	8,3±0,524
Thin part of loop	0,067±0,015	5,63±0,432	20,867±0,827	7,433±0,493
Thick part of loop	0,098±0,057	5,90±0,419	30,467±0,992	7,433±0,493
Distal	0,096±0,056	7,533±0,501	40,967±1,145	11,167±0,578
Nephron (mean)	0,092±0,026	5,817±0,220	26,783±0,466	8,583±0,282
Collecting tubules	0,033±0,033	13,27±0,661	32,367±1,022	9,80±0,569
Nephron + collecting tubules	0,079±0,022	7,307±0,218	27,9±0,424	8,827±0,226

Department of Histology with Embryology and Cytology, Medical Institute, Yaroslavl'. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 81, No. 5, pp. 601-603, May, 1976. Original article submitted May 4, 1975.

TABLE 2. Changes in Relative Number (in %) of Pathological Mitoses (M ± m)

Region of nephron or collecting tubules	Control	3rd day	9th day	15th day
Proximal	0	5,56±2,04	3,63±0,89	9,52±1,86
Thin part of loop	0	4,73±1,63	12,05±1,30	48,65±3,35
Thick part of loop	0	9,60±2,22	2,98±0,56	12,82±2,24
Distal	0	3,54±1,23	2,16±0,41	6,67±1,36
Nephron (mean)	0	5,73±0,87	4,75±0,38	19,73±1,24
Collecting tubules	0	3,52±0,92	3,70±0,61	13,43±1,99
Nephron + collecting tubules	0	4,93±0,65	4,47±0,32	17,67±1,05

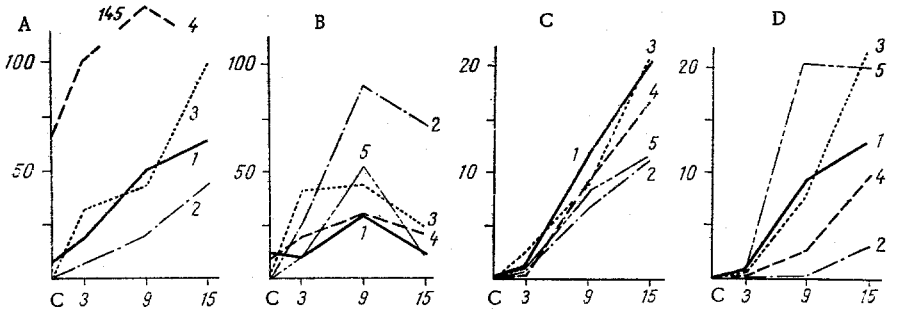

Fig. 1. Changes reflecting damage to and death of epithelium: A) eosinophils; B) pycnosis of nuclei; C) karyorrhexis; D) karyolysis; 1) proximal tubule, 2) thin part of loop, 3) thick part of loop, 4) distal tubule, 5) connecting tubules. Abscissa, control values (C) and time (in days) after crushing of soft tissues; ordinate, number of cells of different types (in $^0/_{00}$ of total number).

TABLE 3. Changes in Mean Area of Nuclei (in μ^2) of Renal Epithelium of Rabbits after Crushing of Soft Tissues (600 nuclei at each time)

Region of nephron or collecting tubules	Time	M ± m	% increase	P
Proximal	Control	26,39±0,24	—	—
	2-nd day	37,64±0,60	42,6	0,001
	4-th day	50,0±0,48	51,4	0,001
	9-th day	53,56±0,49	7,2	0,001
Distal	Control	20,10±0,21	—	—
	2-nd day	29,54±0,39	46,96	0,001
	4-th day	37,62±0,40	40,9	0,001
	9-th day	38,59±0,41	2,6	0,1
Collecting tubules	Control	19,15±0,22	—	—
	2-th day	30,42±0,37	56,4	0,001
	4-th day	36,46±0,40	19,9	0,001
	9-th day	34,38±0,35	—5,7	0,001

Note. 400 nuclei on second day.

[1, 9] of the proximal and distal tubules and of the collecting tubules were measured in the kidneys of 11 rabbits (control, second, fourth, and ninth days) with a planimeter under a magnification of 1450 ×. The dynamics of the cell composition was studied in sections through the kidneys of 12 animals (control, third, ninth, and 15th days). For this purpose 10,000 cells were counted in the convoluted and straight proximal tubules, the thin and thick regions of the loops, the distal convoluted tubules, and the collecting tubules. The number of eosinophils, cells with pycnosis, karyorrhexis, and karyolysis of the nuclei (reflecting injury and death of the epithelium), mitoses (including pathological), amitotic divisions of the nuclei, and binuclear and polynuclear (three nuclei or more) cells (features of regeneration) were counted. The numerical results were subjected to statistical analysis and rank correlation coefficients were calculated between groups of cells found in pathological and reparative reactions.

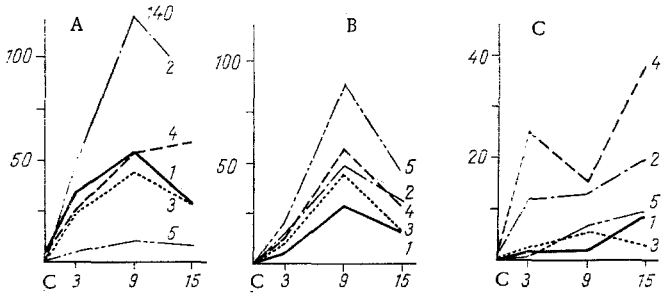

Fig. 2. Changes characterizing regeneration of epithelium: A) amitotic division of nuclei; B) binuclear cells; C) polynuclear cells. Remainder of legend as in Fig. 1.

EXPERIMENTAL RESULTS

Focal disturbances of the hemodynamics in the form of hyperemia, stasis, and hemorrhages, chiefly in the region of the vasa recta of the medulla, were observed for a period of 4 days. Vacuolar and hyaline-droplet degeneration of the epithelium and signs of lysis and necrosis of the nephrons and interstitial tissue were found. Compared with the controls, the number of cells in the experimental animals with various pathological changes was considerably increased (Fig. 1). After 13.5 h karyokinetic figures appeared in the nephrocytes of the thick regions of the loop. The number of mitoses in the epithelium rose sharply until the ninth day, when it was 40.967 $^0/_{00}$ in the distal tubules, and 26.783 $^0/_{00}$ on average for the nephron, after which it fell (Table 1). Nevertheless, even on the 15th day mitotic activity in all parts of the nephron was higher than on the third day. This prolonged maintenance of a high level of cell proliferation was evidently connected with the existence of continuing pathological changes in the epithelium: In nearly all parts the number of eosinophils and of cells with evidence of karyorrhexis and karyolysis was increased (Fig. 1).

After the third day the number of pathological mitoses was considerable (Table 2); these included complete metaphases, deletion of chromosomes, or scattering in this same phase, triplets, multipolar or asymmetrical mitoses, chromatid bridges, etc. [2]. Meanwhile the number of amitotic divisions of the nuclei and of binuclear and multinuclear cells was increased (Fig. 2). On the fifth day, in zones where injury was considerable, there were clear signs of the formation of connective-tissue scars. Calculations showed strong direct positive correlation between the number of cell forms characterizing damage and death of nephrocytes and cells reflecting regeneration in different parts of the nephron, and a moderate degree of similar correlation in the collecting tubules. This indicates that, depending on the severity of injury to a particular part of the nephron, the reparative response will be of comparable intensity. No zones of the kidney were distinguished that could be regarded as the cambium for nephrons or parts of the nephron. An increase also was found in the mean area of the epithelial nuclei (Table 3). This fact is evidently a morphological manifestation of functional compensation and intracellular regeneration [8] and it is connected to some degree with increased mitotic activity of the epithelium [2, 6, 9].

LITERATURE CITED

1. G. G. Avtandilov, Morphometry in Pathology [in Russian], Moscow (1973), pp. 152-171.
2. I. A. Alov, Cytophysiology and Pathology of Mitosis [in Russian], Moscow (1972).
3. M. G. Danilov, A. N. Chistovich, and V. G. Chudakov, in: Functional and Morphological Changes during Prolonged Crushing of the Tissues [in Russian], Leningrad (1959), pp. 18-65.
4. M. I. Kuzin, Clinical Features, Pathogenesis, and Treatment of the Prolonged Crushing Syndrome [in Russian], Moscow (1959).
5. N. N. Kochetov, "Changes and regenerative processes in organs after orthostatic collapse," Graduation Lecture [in Russian], Yaroslavl' (1973).
6. L. D. Kozner and L. M. Farutina, in: Compensatory Hypertrophy of Organs of Mammals and Man [in Russian], Moscow (1963), pp. 28-80.
7. L. D. Kozner, Arkh. Pat., No. 7, 66 (1974).
8. D. S. Sarkisov, Regeneration and Its Clinical Importance [in Russian], Moscow (1970).
9. Ya. E. Khesin, Dimensions of the Nuclei and the Functional State of Cells [in Russian], Moscow (1967).