CHANGE IN THE SIZE OF THE NUCLEUS AND BODY OF CELLS IN A RAT'S REGENERATING KIDNEY

G. G. Samsonidze

From the Laboratory of Growth and Development (Head – Prof. L. D. Liozner) of the Institute of Experimental Biology (Dir. – Prof. I. N. Maiskii), AMN SSSR, Moscow and the Department of Histology of Tbilisi Medical Institute
(Presented by AMN SSSR Active Member A. V. Lebedinskii)
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In previous papers [2, 3, 4], we showed that restorative processes typical of regenerative hypertrophy [2] occur after total excision of one kidney and excision of $1/3$ to $1/2$ of the other kidney in rats. The regenerated kidney is much bigger and heavier than the original one; hypertrophy is observed in every part of the nephrons, and cell hyperplasia and hypertrophy are observed.

The purpose of this work was to determine how the nucleus and cytoplasm of the cells change during regeneration and the effect of the increased functional load on their size.

The literature data concerning changes in the kidney cells observed during the compensatory hypertrophy following unilateral nephrectomy are contradictory [1, 5 - 8]. A number of authors [6, 7] doubt that any stable increase occurs in the size of the nucleus during compensatory hypertrophy. We anticipated more definite results in out experiments due to the fact that hypertrophy is more pronounced during kidney regeneration than after unilateral nephrectomy.

EXPERIMENTAL

The experiments were performed on male white rats weighing 130 - 390 g. We performed an operation on the experimental animals totally excising the left kidney and resecting $1/3 - 1/2$ of the right. The material used for examination was taken from animals killed 1, 2, 5, 17, 23, 25, 35, 59 and 89 days after the operation, eight rats being used for each interval. Twenty rats were used as a control, ten of which were sacrificed at the beginning of the experiments (initial control) and ten at the end (final control). Bouin's fluid and a 10% formalin solution were used to fix the kidneys. The pieces were embedded in paraffin, and the sections were stained with hematoxylin according to Bemer and with azocarmine and hematoxylin according to Heidenhain.

A screw ocular micrometer was used to measure the diameter of the nuclei in the outer layer of Bowman's capsule and the convoluted and collecting tubules. The formula for the ellipse was used to compute the area of the nuclei: S of nucleus = $\frac{\Pi Dd}{4}$, where $\frac{\Pi}{4}$ = 0.785, D = the large diameter of the nucleus and $\underline{d}$ = the small diameter. An Abbe drawing apparatus was used to draw in outline cross sections of the kidney tubules (magnified 900×) and to make outline drawings of the nuclei of the tubule cells on paper of a constant thickness. The outlined shapes were then cut out and weighed on torsion scales. Then, knowing the weight of the paper and the magnification, we computed the true values for the area of the tubule occupied by the cells and the area of the nucleus. By subtracting the area of the nucleus from the area of the tubule occupied by cells and dividing the value obtained by the number of nuclei, we obtained the area of the cytoplasm in a single cell. We also computed the nucleus-cytoplasm relationship.

The average from 50 measurements was used to compute each of the above indices. Statistical processing showed the results obtained from this number of measurements to be constant.

RESULTS

Table 1 presents data showing the change in the average area of the nuclei in the external layer of Bowman's capsule and in the convoluted and collecting tubules.

As Table 1 shows, there was no change in the average area of the nuclei in the external layer of Bowman's capsule in the intact kidney during the experimental period. In the experimental animals, this index increased soon after the operation, being highest on the 23rd day. The increase observed in the area of the nucleus (14%) approximated the statistically significant (D = 0.03). Since concurrent changes were observed in the nuclei in the other parts of the nephron, there is reason to believe that the increases detected were more than chance fluctuations. Subsequently, the average area of the nuclei decreased until it approximated the control data 89 days after the operation.

TABLE 1. Change in the Area of Cell Nuclei in the Kidneys of Experimental and Control Rats

Number of days after operation	Average area of nucleus (in μ^2): in external layer of Bowman's capsule	in convoluted tubule	in collecting tubule: of cortex	of medulla
1	12,5	24,8	20,9	24,4
2	12,7	24,9	21,4	25,7
5	12,9	25,2	21,8	26,5
17	13,0	29,5	25,1	29,7
23	13,7	31,0	26,3	30,2
35	13,2	29,2	24,5	28,5
59	12,5	27,9	23,3	25,5
89	12,3	28,0	19,8	20,4
0 (initial control)	12,2	21,5	18,6	18,4
89 (final control)	12,0	20,8	18,1	18,3

Although there was no change in the size of the nucleus in the convoluted tubule in the intact kidney of the control rats, the average area of the nuclei in this portion of the nephron increased in the operated animals from the very beginning of the experiment, reaching its highest point towards the end of the first month after the operation. After 23 days, the area of the nucleus had increased 49% of the final control (the values obtained all conformed to this figure). The area of the nuclei decreased somewhat thereafter, but remained greater than in the control.

In the collecting tubules of the cortex and medulla, the average area of the nuclei changed in a similar fashion, but the decrease in the size of the nucleus observed at the later stages of regeneration was less pronounced. Eighty-nine days after the operation, the nuclei in the collecting tubules approximated those in the control animals in size (the differences detected were not statistically significant).

As Table 2 shows, the average area occupied by cells in the cross sections of the convoluted tubule and of the cortical and medullary collecting tubules increased on all these cross sections. Analogous data was obtained in a previous work in which another method of measuring was used [3].

The area occupied by cytoplasm, however, only increased in the convoluted tubules and in the collecting tubules of the cortical substance. In the collecting tubules of the medullary substance, the area occupied by cytoplasm was even smaller than in the control animals, although the differences were within the margin of incidental fluctuation.

TABLE 2. Changes in the Area of Nuclei and Cytoplasm in Kidneys of Experimental and Control Rats 23 Days after Operation

Object examined	Animal group	Area (in μ^2): occupied by cells	occupied by cytoplasm	occupied by nuclei	Number of nuclei	Area (in μ^2): cytoplasm in a single cell	nucleus of a single cell	Nucleus cytoplasm relationship
Convoluted tubule	Control, experimental	695,3 1071,7	581,4 863,7	110,9 208,0	5,0 7,3	94,3 119,7	21,9 28,8	0,23 0,24
Collecting tubule of cortical substance	Control, experimental	468,0 686,5	369,9 520,0	90,7 166,5	5,2 6,3	71,7 81,0	17,6 25,7	0,23 0,32
Collecting tubule of medullary substance	Control, experimental	425,7 482,7	345,3 334,1	80,4 148,6	4,4 5,3	77,6 62,5	18,0 27,8	0,23 0,45

The cross sections of the tubules showed that the area occupied by nuclei increased in both the convoluted tubules and the collecting tubules of the cortical and medullary substances. This index increased 87.6% in the convoluted tubule, 83.6% in the collecting tubule of the cortical substance and 84.8% in the collecting tubule of the medullary substance. This increase was found to be statistically significant.

The increase in the number of nuclei observed on the tubule cross sections agreed with data obtained in a previous work [3]. There was no change between the number of nuclei in the experiment and in the control. Further computations showed that the changes in cytoplasm area of the individual cells during the process of regeneration were variable. A statistically significant increase of 27% in the area of cytoplasm was observed in the convoluted tubules. There was a 13% increase in the area of cytoplasm in individual cells of the cortical collecting tubule, but this increase was not statistically significant. In the collecting tubules of the medullary substance, the area of cytoplasm in individual cells decreased 19.5%, and there was no change between the experimental and control levels.

The data showing the change in the nuclear area of individual cells approximated those obtained by measuring the nuclei with a screw ocular micrometer (i.e., the data given in Table 1), which confirms the reliability of our measuring methods.

The nucleus-cytoplasm relationship in the cells of the convoluted tubules hardly changed at all during the regeneration process, due to the proportional increase in the nuclear and cytoplasmic areas. However, this index increased greatly in the collecting tubules, especially in the medullary substance. This was due to the failure of the cytoplasm, which sometimes even decreased in area, to keep pace with the growth of the nucleus. Several works [7] indicate that the changes which occur in the convoluted tubules during compensatory hypertrophy differ in nature from those which occur in the collecting tubules. The difference in the reactions of the cells in the convoluted tubules and those in the collecting tubules to the excision of a large amount of renal tissue is connected with the difference in their functions. The main function of the collecting tubules, for example, is to excrete urine, and intensification of this function evidently causes the cells lining these tubules to decrease in size rather than to increase.

Our data, therefore, corroborate the authors who found an increase in the size of cells and nuclei during compensatory hypertrophy of the kidney and who emphasized the stable character of this phenomenon. In experiments with kidney regeneration, cell and nuclear hypertrophy was very clearly expressed in certain portions of the kidney and lasted a long time (at least 3 months). The changes are variably expressed, however, in the different portions of the kidney. Although a high level of nuclear and cytoplasmic hypertrophy is observed for a long period in the convoluted tubules, in other parts of the nephron, nuclear hypertrophy is less pronounced (Bowman's capsule) or lasts a relatively short time. In the collecting tubules, the cytoplasm not only does not hypertrophy, but even decreases in area.

During regeneration, therefore, as during compensatory hypertrophy, diverse changes occur in the kidney. In the convoluted tubules, hypertrophy of the nucleus and cytoplasm is particularly pronounced and is retained for a long time.

SUMMARY

The area of the nuclei was studied in the external layer of Bowman's capsule, convoluted tubules and collecting tubules of the regenerating kidney in rats after the removal of one kidney and resection of $^1/_3$ and $^1/_2$ of another one. Soon after the operation the area of the nuclei increases in all the parts of the nephron, reaching the maximum on the 23rd day. In the convoluted tubules hypertrophy of the nuclei is retained for a period of 89 days, whereas in the rest of the portions— it disappears. The area of the cytoplasms in the regenerating kidney is seen to be increased in the cells of the convoluted tubules and decreased in the collecting tubules of the medullary layer on the 23rd post-operative day. The nuclear-plasm relationship is within the normal range in the convoluted tubules and is markedly increased in the tubules.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.
