CELLULAR ACTIVATION FOLLOWING INJURY OF THE KIDNEYS

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During the reparative regeneration of various organs one notes an increase in the cellular content of RNA and DNA, hypertrophy of the cells, and an increase in their multiplication. Cell division, in this case, rises both in the organs with high mitotic activities and in the organs where the number of mitoses is minimal or where such activity is completely absent. A study of regeneration in the liver and salivary glands has indicated that cellular activation during regeneration is related to the action of protein products from tissue breakdown [1-4]. The introduction of liver and salivary gland extracts caused an increase in the nucleic acid concentration within the cells, cellular hypertrophy, and activation of cell division. To a greater or lesser degree this reaction was organ specific in character. Histochemical changes and cell growth preceded a wave of mitoses. Activation of the cells by the products of tissue breakdown is apparently accomplished through the nucleic acid metabolism of the cell [2, 3]. It could be postulated that cellular activation during the regeneration of other organs was related to the same mechanism.

We undertook experiments in which we studied cellular activation following injury of the kidneys.

METHOD

The experiments were carried out on white mice 2.5-3 months of age. In the first series of experiments the animals were subjected to unilateral partial extirpation of the kidney; approximately a third to a half of the organ was removed. Mice from the same litter and kept under the same conditions, but notoperated upon, served as the control. The animals were sacrificed at the same time—3 days after the operation. Histological investigation was carried out on the regenerating (right) and intact (left) kidneys. In the second series of experiments we studied the changes in the kidneys following the introduction of protein extracts from various organs. The organs were ground up, extracted with a sodium chloride solution for 1.5 hours under refrigeration, and centrifuged. The extract (0.5-1% protein) was injected into the white mice intraperitoneally, using 0.2 ml per animal over the course of three days. The material was fixed 3 days after the last injection. In the third series of experiments we studied the changes in the left kidney after preliminary complete removal of the right kidney. Both the test animals and the controls in all series of the experiments were sacrificed at the same time of day—4 P.M.

In all the series of experiments we established the mitotic activity in the primary divisions of the kidney, the dimensions of the cells and their nuclei, and the cellular concentration of RNA and DNA. To determine the mitotic activity we counted the number of dividing cells in a constant area, set at 1.65 mm^2 (in the liver this area was 8.55 mm^2). At the same time we calculated the phase coefficient (proportion of early phases of division to late). The areas of the cells and their nuclei in the cross section were measured by means of a projected drawing of the cells and their planimetry. Two hundred cells were measured in each operated or intact kidney examined. In each group of animals (test and control) the determinations were performed on the kidneys of 2 mice.* In addi-

* For purposes of simplification the average of the dimensions in the two animals is presented in tables (400 cells).

tion to the mitotic activity and the dimensions of the cells, the cellular concentration of RNA and DNA was established in each experiment. The material was fixed in Helly's solution. RNA was demonstrated by staining with methyl green and pyronine; Feulgen's reaction was used for DNA.

RESULTS

In the primary divisions of the kidney of white mice only isolated dividing cells were encountered. In a number of cases we did not observe any mitoses at all. After partial extirpation of the kidney the mitotic activity in the remainder of the organ increased. On the third day following the operation the number of dividing cells exceeded the level of mitotic activity in the control animals by 5 times. An increase in the dimensions of the cells and their nuclei was observed (Table 1).

TABLE 1

Changes in the Mitotic Activity of the Renal Epithelium during Wound Healing and following the Administration of an Extract of This Organ

Group of animals	No. of animals	Number of mitoses (M ± m) and phase coefficient (k)	Area in μ^2 (M ± m)	
			cells	nuclei
Control	6	1,1±0,43; κ =0,6	63,45±0,61	9,99±0,15
Test: injured kidneys	11	5,5±0,23; κ =4,05	80,92±0,77	12,66∓0,16
intact kidney	11	3,2±0,4; κ =2,1	74,1±0,76	11,18±0,16
	—	$P \ll 0,01$	$P<0,0001$	$P<0,0001$
Control	10	2,8±0,4; κ =1,7	74,9±0,9	14,1±0,17
Test: administration of extract	9	8,5±1,5; κ =3,7	103,3±1,3	17,9±0,2
	—	P=0,002	$P<0,0001$	$P \ll 0,0001$

TABLE 2

The Effect of the Extract of Various Organs on the Mitotic Activity

Group of animals	No. of animals	Number of mitoses (M ± m) and phase coefficient (k)		
		in the kidney	in the cornea	in the liver
Control . . .	10	2,8±0,46; κ =1,7	55,5±8,2; κ =0,9	—
Test: injection of renal extract	10	8,5±1,53; κ =3,7	54,3±9,6; κ =0,9	—
Control	7	0,57±0,29; κ =0,4	—	0
Test: injection of liver extract	7	0,57±0,29; κ =0,5	—	2,3±0,3; κ =1,8
Control	6	0,66±0,1, κ =0,3	—	—
Test: injection of pancreas extract	6	0,5±0,2; κ =0,5	—	—

Just as in the case of the regenerating salivary gland [4], in the injured kidney the number of small cells decreased, the number of large cells increased, and very large cells appeared which were not encountered in the control animals. An increase in the dimensions of the cells and their nuclei in the injured kidney were clearly shifted to the right (Fig. 1). As in the other organs, the reaction of the kidney to injury was accompanied by ele-

vation of the cellular concentration of RNA and DNA. These changes, however, were less impressive than in comparable experiments on the salivary glands.

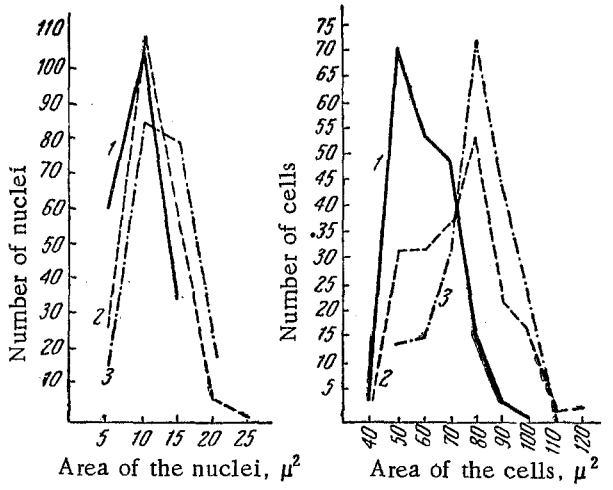

Fig. 1. Distribution curves for the dimensions of the cells and their nuclei in the kidney after injury. 1) Control; 2) intact kidney; 3) injured kidney.

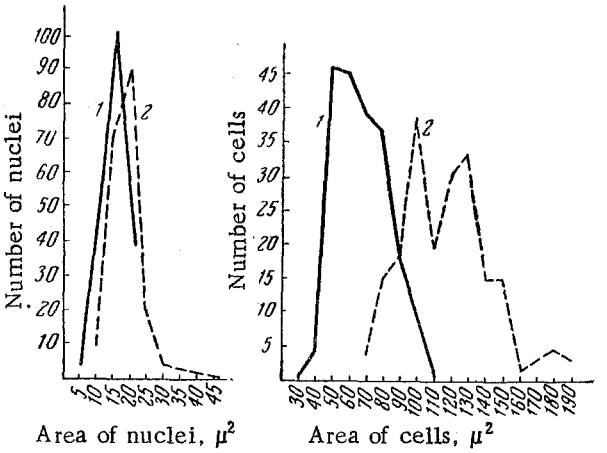

Fig. 2. Distribution curves for the dimensions of the cells and their nuclei in the kidney after injection of extract. 1) Control; 2) after injection of renal extract.

The "overflow" nature of this reaction suggests a humoral mechanism for cellular activation during regeneration. Study of the intact kidney showed that with injury of one of the kidneys the same changes occur in the second. In the undisturbed kidney we observed an increase in the mitotic activity and in the dimensions of the cells and their nuclei, as well as an elevation in the cellular concentration of DNA and RNA (see Table 1). These changes were of lesser magnitude than in the injured organ, but were clearly manifested in all the experiments. Cellular activation extended not only to the injured organ, but to the intact one as well.

The experiments on the liver and salivary glands showed that cellular activation during regeneration of these organs is connected with the action of the protein products from the tissue breakdown that arises secondary to injury. In line with this we carried out a second series of experiments, in which the white mice were injected with renal extract. On the third day after injection of the extract a clearly observable increase in the mitotic activity was observed in the intact kidneys, and the dimensions of the cells and their nuclei were seen to grow larger (Table 2, Fig. 2). An elevation in the intensity of the cellular reaction for RNA and DNA was also observed. Injection of the organ extracts thus reproduced the same changes as were observed during wound healing of the kidney. The results of these experiments, together with the data obtained earlier, justify postulating that activation of the division and growth of cells during regeneration is related to the action of the protein products from the breakdown of injured tissues. The experiments with injection of the extracts from various organs testify to the organ specificity of the activating effect attributable to the products of tissue breakdown. While the kidney extract injections caused a clear increase in the mitotic activity and the dimensions of the cells of that organ, similar changes were not observed in the kidney following the use of extracts of the liver and pancreas (see Table 2). At the same time, injections of kidney extract only elevated the mitotic activity in that organ, and did not alter it in the cornea, while liver extract increased the mitotic acitivity in the liver and did not alter it in the kidney.

The action of the products of tissue breakdown were apparently not the only factor in the cellular activation during wound healing in the kidney. Under conditions of compensatory hypertrophy of the kidney due to the removal of the other organ analogous changes were noted in the mitotic activity, the concentration of nucleic acids in the cells, and the dimensions of the latter [6, 7]. In connection with this we set up a third series of experiments, in which we studied the changes in the kidney following removal of the contralateral kidney without its injury; therefore, we excluded the possibility of the action of the tissue breakdown products. The results of the experiments (Table 3, Fig. 3) showed that in the remaining kidney the mitotic activity rose and the dimensions of the cells increased.

TABLE 3

Changes in the Mitotic Activity and Dimensions of the Cells in the Kidney following Complete Removal of the Other Kidney

Group of animals	No. of animals	Time elapsed after operation days	No. of mitoses (M ± m) and phase coefficient (k)	Area in μ^2 (M ± m)	
				cells	nuclei
Control	8	—	1,5±0,6; κ =1,3	74,95±0,56	11,34±0,14
Test: removal of kidney	12	3	2,0±0,6; κ =1,5	90,8±0,9	14,51±0,26
	—	—	P=0,3	P <0,0001	P <0,0001
Control	8	—	1,75±0,2; κ =0,6	71,32±0,82	11,25±0,13
Test: removal of kidney	8	7	9,4±0,05; κ =3,1	97,75±0,89	14,1±0,14
	—	—	P< 0,001	P <0,0001	P <0,0001

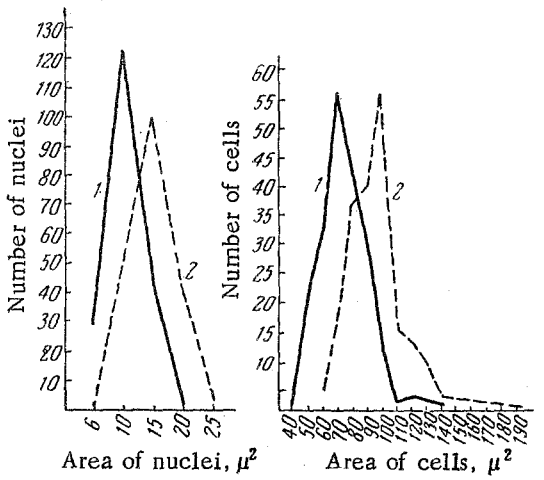

Fig. 3. Distribution curves for the dimensions of the cells and their nuclei in the kidney after removal of one kidney. 1) Control; 2) complete removal of one kidney (7-day interval).

However, these changes ensued later than under the conditions of the injured kidney. Three days after the complete removal of one of the kidneys the mitotic activity in the second kidney did not change, and only hypertrophy of the cells was noted. Only on the seventh day in our experiments did we observe a clearly defined stimulation of the mitotic activity. Following partial removal of a kidney, when the action of the tissue breakdown products was retained, the mitotic activity in the remainder of the injured kidney and in the intact organ was already increased by the 3rd day (see Table 1).

The results of these experiments therefore show that cellular activation during wound healing in the kidney may be related not only to the action of tissue breakdown products, but also to the phenomena of compensation, which are probably a result of the augmented functioning of the remaining portion of the organ. The influence of this second factor on the activation appears at later stages, when the functioning of the injured organ is restored. The importance of compensatingly elevated functioning in the process of regeneration is emphasized by data which indicate that a rising functional load on the liver accelerates the regeneration of this organ [5].

The observations presented support the hypothesis that the appearance of cell divisions and hypertrophy of the cells following the removal of a portion of the kidney are connected with activation of the cells by protein products of tissue breakdown and with compensatory phenomena in the injured organ. Activation of the cells by the tissue breakdown products is organ specific in nature, and is apparently accomplished through the nucleic acid metabolism of the cells. The functioning of the organ, compensatingly elevated in the course of a long period of time, is apparently a second factor in the cellular activation, its effect appearing at later stages.

SUMMARY

On the third day after partial unilateral extirpation of the kidneys, there was a rise of mitotic activity, hypertrophy of the cells and their nuclei and a rise of the RNA and DNA content in the cells. Cellular activation during healing of the kidney wound occurred not only in the regenerating organ, but also in the intact one on the contralateral side. Activation of the cells in wound healing is connected with the action of products of tissue protein disintegration. Administration of a renal tissue extract caused a marked increase of mitotic activity, hyper-

trophy of the cells and a rise of nucleic acid content. This reaction is organ specific in character. The renal extract stimulated the mitotic activity of the kidney alone, whereas hepatic and pancreatic extracts had no effect on this activity in the kidney.

The second factor involved in cellular activation during wound healing was the compensatory increase of the organ function. With complete removal of one of the kidneys, an increase of mitotic activity and cellular hypertrophy and a rise of the RNA and DNA content were noted in the intact kidney. The effect of the second activation factor was manifested at a later stage, when the function of the injured organ was returning. With complete removal of one of the kidneys, a rise of mitotic activity in the intact kidney became manifest only on the 7th day after the operation.

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