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PROLIFERATIVE ACTIVITY IN SOME TISSUES OF MICE UNILATERALLY NEPHRECTOMIZED AT DIFFERENT TIMES OF DAY

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Unilateral nephrectomy was performed on mice at different times during the morning and evening. Mitotic activity was investigated in the proximal portions of the convoluted tubules of the nephron of the remaining kidney and in the corneal epithelium 48 and 60 h after the operation. If the operation was performed in the morning the mitotic index (MI) was 4.3 times higher than the control and still remained high 60 h after the operation. If the operation was performed in the evening MI was 9.3 times higher than the control and fell after 60 h. It is concluded that the cells of the renal epithelium in animals nephrectomized in the evening divide more synchronously than in mice nephrectomized in the morning. Nephrectomy did not affect the level or rhythm of cellular proliferation in the corneal epithelium.

KEY WORDS: unilateral nephrectomy; mitotic index; diurnal rhythm; cornea.

The presence of a diurnal rhythm of cell division in normal [2] and regenerating organs [3, 4, 8] is a firmly established fact. A problem which has arisen in the course of experiments to study regeneration is whether the time of day at which an organ is resected affects changes in mitotic activity, or whether the mitotic activity of regenerating organs is determined entirely by the time elapsing after the operation, i.e., does not obey the rules of diurnal rhythms. Neither in experiments to study regeneration of the liver [3] nor in experiments to study compensatory hypertrophy of the kidney has an unambiguous answer to this question been obtained. Mitotic activity in the liver after removal of two-thirds of the organ reaches a maximum as a rule in the morning [4], although the time elapsing after the operation is important. Other workers consider that during regeneration of the liver the time elapsing after the operation plays the principal role [6, 9].

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TABLE 1. Mitotic Activity in Proximal Portions of Convoluted Tubules of Nephron and in Corneal Epithelium of Mice at Different Times after Unilateral Nephrectomy

Tissue	Time of operation	MI 48 h after operation, ‰			MI 60 h after operation, ‰		
		control	experiment	P	control	experiment	P
Epithelium of renal tubules	11 a.m.-2 p.m. (group 1)	0,40±0,13 (n=6)	1,74±0,48 (n=6)	0.022	0,39±0,15 (n=6)	1,81±0.68 (n=6)	<0.05
Corneal epithelium		14.40±1,3 (n=6)	15,0±1,75 (n=6)	—	6,5±0,99 (n=6)	5,8±0.7 (n=6)	—
Epithelium of renal tubules	7-11 p.m. (group 2)	0.25±0,08 (n=6)	2.32±0.67 (n=6)	0.008	0,40±0,13 (n=6)	0,91±0.14 (n=6)	0,02
Corneal epithelium		7.1±0.6 (n=6)	8,9±1.2 (n=6)	—	14,40±1.30 (n=6)	15.2±2.2 (n=6)	—

Legend. Number of animals in parentheses.

As regards the kidney undergoing hypertrophy after unilateral nephrectomy, the relations between the above-mentioned temporal parameters have received even less study. Sharipov [5] found that the peak of mitosis in the residual fragment of the kidney after removal of the whole of one kidney and resection of part of the other was shifted depending on the time of the operation.

In view of the importance of this problem, it was decided to continue the investigation of cell proliferation during compensatory hypertrophy by using as a test object the mouse kidney, not hitherto studied from this aspect.

Mitotic activity was determined in the mouse kidney after unilateral nephrectomy performed at different times of day, and also in the corneal epithelium, with no functional connection with the kidney.

EXPERIMENTAL METHOD

Experiments were carried out on 42 noninbred male mice weighing 21-25 g. Eighteen mice served as the control. The experimental animals were divided into two groups. In the mice of group 1 the left kidney was removed at 11 a.m.-2 p.m. and the animals were killed 48 h and 60 h after the operation. Nephrectomy was performed on the mice of group 2 at 7-11 p.m. and these mice also were killed 48 and 60 h later. The operations were performed under pentobarbital anesthesia (0.03 ml of a 2% solution/10 g body weight). The choice of the time of sacrifice was determined by the fact that a sharp rise in the number of mitoses and in the number of cells synthesizing DNA takes place in the kidney 48 h after unilateral nephrectomy [10, 12]. The number of mitoses was counted in the proximal portions of the convoluted tubules of the nephron by examination of 25,000-35,000 cells, and in the corneal epithelium by counting 20,000 cells. The mitotic index (MI) was expressed in pro mille. The numerical results were subjected to statistical analysis by the Fisher–Student method and by means of the Wilcoxon–Mann–Whitney nonparametric criterion (U).

EXPERIMENTAL RESULTS

The experimental results are given in Table 1. They show that 48 h after the operation performed in the morning, MI in the residual kidney was increased by 4.3 times ($P = 0.022$) and it remained at the same level 60 h after the operation, i.e., during the evening. When the kidney was removed in the evening, the increase in MI 48 h later was greater, mainly 9.3 times the control level. Incidentally, Heine et al. [7] also found a higher index of labeled nuclei and a higher mitotic index in the rat kidney 48 h after unilateral nephrectomy performed at 7 p.m. compared with the corresponding time after an operation at 7 a.m.

MI in the proximal portions of the renal tubules 60 h after the operation (i.e., after sacrifice in the morning) was reduced by 2.5 times ($P < 0.05$) compared with the previous period of the experiment.

The results thus show that, regardless of the time of the operation, an increase in mitotic activity is observed in the mouse kidney 48 h later. Meanwhile, definite differences exist between the results of operations performed at different times of day. Unilateral nephrectomy performed in the morning led to prolonged elevation of mitotic activity in the residual kidney, of the same level in the morning and evening. An operation performed in the evening gave different results. An increase in MI 48 h after the operation was followed by a decrease 60 h after the operation, i.e., in the morning. Differences in the MI values also were significant when

the results for the mice of groups 1 and 2, killed in the morning (1.74 and 0.91% respectively; $P < 0.05$) were compared. In the kidney hypertrophied after unilateral nephrectomy, the principal role in the formation of the wave of mitosis is thus played by the time elapsing after the operation. The time of the operation evidently affects the synchronization of the entry of the cells into mitosis after the operation. In animals nephrectomized in the evening, cells of the renal epithelium divide more synchronously than in mice nephrectomized in the morning. These observations agree, as regards both the main conclusion and also the character of the fluctuations in MI depending on the time of day, with those of Saetren [11], who removed one kidney and resected half of the other kidney in rats. He found that after an operation at 9 p.m. the number of mitoses rose rapidly and fell rapidly. After an operation at 9 a.m. the number of mitoses increased more slowly and the curve of mitotic activity was more protracted.

Vinogradova showed [1] that removal of two-thirds of the liver in mice increases the intensity of physiological regeneration in the corneal epithelium. It was interesting to discover how unilateral nephrectomy affects the level of cell proliferation in the corneal epithelial tissue, functionally unconnected with the kidney. The results of determination of MI in the corneal epithelium of the control and experimental animals showed no difference between the corresponding values, and MI in both the control and the experimental series was always higher in the morning than in the evening. Nephrectomy and trauma (operation) at the times studied thus do not affect the rhythm and intensity of cell division in the corneal epithelium.

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