REPAIR PROCESSES IN THE LIVER AND ADRENAL CORTEX OF RATS AFTER AN EXPERIMENTAL VASCULAR CRISIS

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Repair processes in the liver and adrenal cortex of hemiadrenalectomized female rats with an acute disturbance of the circulation resulting from orthostasis were studied by quantitative methods (cytometry, karyometry, nucleolometry, determination of the cell composition and of the nucleo-cytoplasmic and nucleolo-nuclear ratios). Regeneration of the organs studied showed some common features. During the first days after the experiment the number of dying cells in the organs was increased. Meanwhile the number of amitoses and of binuclear cells increased in the liver. By the 15th day in the adrenal and by the 45th day in the liver the cell composition of the organs was stabilized. During the first week after orthostasis mitotic activity of the epithelial cells was increased in both organs. In the later stages regeneration took place at the intracellular level (activation of the nuclear apparatus and hypertrophy of the cytoplasm of the cells).

KEY WORDS: rat liver and adrenal cortex; orthostasis; regeneration; morphometry.

In human pathology lesions of the internal organs arising as a result of acute disturbances of the circulation are not infrequently found [1, 2, 5]. However, repair processes after lesions of this type have been inadequately studied, especially from the quantitative aspect.

The object of this investigation was to study the dynamics of the morphometric indices characterizing injury and repair processes in the liver and adrenal cortex of rats after an experimental vascular crisis.

EXPERIMENTAL METHOD

The experimental model of an acute circulatory disturbance arising as a result of fixation of albino rats, weighing 180-230 g, in the vertical position with the head uppermost for 10-12 h was used in the experiments. With the object of reducing the resistance of the animals, left-sided adrenalectomy was performed on them 14-15 days before the experiment. The animals were decapitated at times ranging from 9 h to 90 days after the beginning of orthostasis, always in the morning. Pieces of liver from 96 rats and of adrenals from 50 rats from this group were fixed in Bouin's fluid. Organs of intact animals and also of hemiadrenalectomized rats, 15, 45, and 60 days after the operation, served as the control. Epithelial cells were counted (12,000-15,000 cells at each time in the liver and 5,000-6,000 cells in the adrenal) in the liver 1, 3, 5, 12, 20, 45, and 90 days after orthostasis, and in the adrenals at the boundary between the zona glomerulosa and zona fasciculata (24 rats) 1, 5, 7, 9, 15, and 30 days after orthostasis. Cell forms reflecting injury and death of the cells – cells with pycnosis and with lysis of the nuclei [3], and also processes of repair (mitosis, amitotic division of the nuclei, binuclear cells in the liver; mitosis, large hyperchromatic nuclei, and cells with expulsion of the nucleolus from the nucleus in the adrenals) were distinguished. Changes in the mean size of the cells were judged from the dynamics of their number per field of vision of the microscope. The mean area of the cells with morphologically unchanged nuclei also was determined in the control and at the later stages (30-45 days) by a photogravimetric method (magnification 1,300×). For karyometry and nucleolometry a special ocular scale [4] was used. At each time 400 cells in the liver and 150-200 cells in the adrenal were measured. The results were subjected to statistical analysis.

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TABLE 1. Morphometric Characteristics of Cells of the Liver and Zona Fasciculata of the Adrenal Cortex in the Control and in the Late Period after Orthostasis (M ± m)

Organ studied and time of investigation	Mean area of cell	Mean area of nucleus	Mean area of cytoplasm	Nucleo-cytoplasmic ratio, %	Mean number of nucleoli in nucleus	Mean area of nucleolus, μ^2	Nucleolo-nuclear ratio, %
	μ^2						
Mononuclear cells							
Liver							
Control (60 days after hemiadrenalectomy)	221,55±3,69	40,83±0,39	180,72±4,08	22,59±0,74	1,478	2,164±0,056	7,83±0,28
45 days after orthostasis	267,22±12,49	46,88±0,27	220,34±12,76	21,28±1,43	1,930	2,190±0,061	9,02±0,30
P	<0,01	<0,001	<0,05	>0,05		>0,05	<0,01
Binuclear cells							
Control							
45 days after orthostasis	297,05±11,86	36,12±0,31	224,81±12,48	32,13±2,18	1,458	2,011±0,050	8,12±0,27
P	348,18±13,72	39,79±0,32	268,60±14,36	29,63±1,92	1,970	1,948±0,045	9,64±0,31
Adrenal	<0,05	<0,001	<0,05	>0,05		>0,05	<0,01
Control 1 (intact rats)	206,55±3,82	26,20±0,41	180,35±4,23	14,53±0,57	1,631	0,785±0,384	4,89±2,50
Control 2 (45 days after hemiadrenalectomy)	241,84±9,20	31,33±0,50	210,51±9,70	14,88±0,97	1,822	0,785±0,330	4,57±2,03
P_1	<0,05	<0,001	<0,05	>0,05		0	>0,05
30 days after orthostasis	307,56±12,32	31,41±0,65	276,15±12,97	11,37±0,81	1,952	3,142±1,322	19,5±8,86
P_2	<0,05	>0,05	<0,05	<0,05		<0,1	<0,1

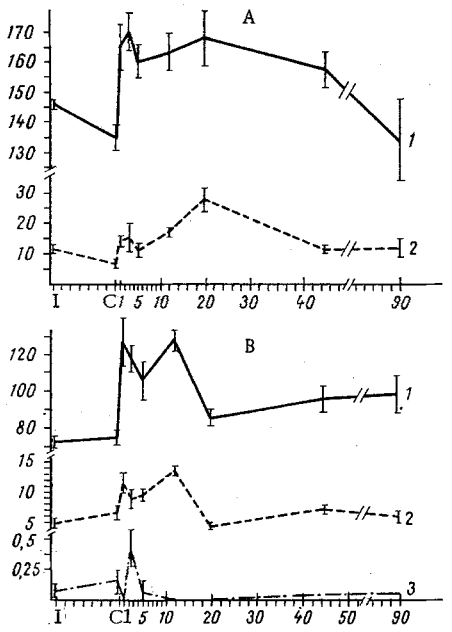

Fig. 1

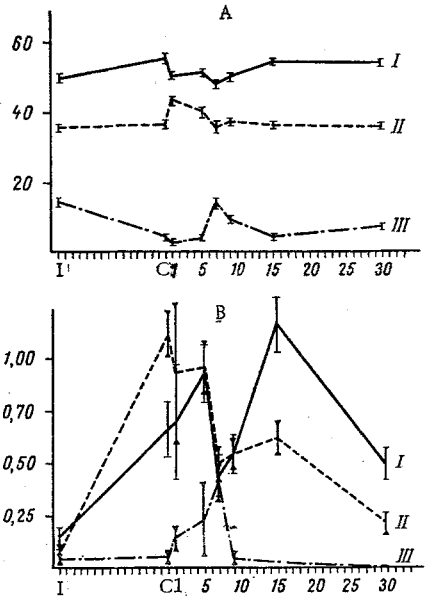

Fig. 2

Fig. 1. Numbers of different cell forms in liver in control and at various times after orthostasis. Abscissa, time after orthostasis (in days); ordinate, number of cells (in ‰). A: 1) cells with karyolysis, 2) cells with karyopycnosis; B: 1) binuclear cells, 2) cells with amitotic division of nuclei in progress, 3) mitoses. I) intact organ; C) liver 15 days after hemiadrenalectomy.

Fig. 2. Number of different cell forms in zona fasciculata of adrenal cortex in control and at different times after orthostasis. Abscissa, time after orthostasis (in days); ordinate, number of cells (in %). A: I) cells with morphologically unchanged nuclei, II) cells with karyolysis, III) cells with karyopycnosis; B: I) cells with large hyperchromatic nuclei, II) cells with nucleolus expelled from nucleus, III) mitoses. I) Intact organ; C) adrenal 15 days after hemiadrenalectomy.

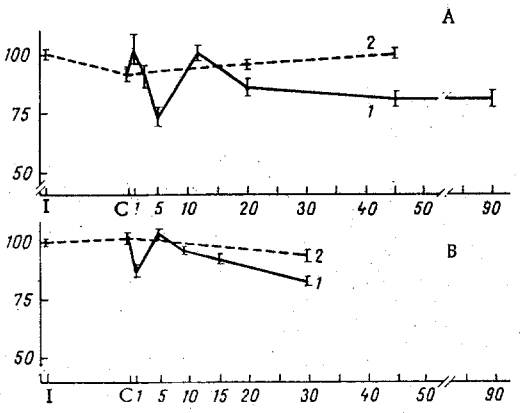

Fig. 3. Changes in mean number of cell forms per field of vision of microscope in liver (A) and adrenal cortex (B). Abscissa, time after orthostasis (in days); ordinate, number of cells per field of vision of microscope (in % compared with intact organs). 1) Experiment; 2) control (hemiadrenalectomized animals not subjected to orthostasis). I) Intact organ; C) 15 days after hemiadrenalectomy.

EXPERIMENTAL RESULTS

To assess the changes taking place as a result of orthostasis, they had to be distinguished from the effect of hemiadrenalectomy on the structure of the two organs studied. In the adrenal cells expulsion of the nucleolus from the nucleus was much more frequently found 15 days after the operation, whereas the number of pycnotic cells was reduced ($P < 0.05$). By the 45th day compensatory hypertrophy of the nucleus and cytoplasm had developed in the cells of the zona fasciculata (Table 1). In the liver by the 15th day the number of cells with pycnosis and karyolysis was reduced statistically significantly ($P < 0.05$). By the 60th day all the morphometric indices were at the characteristic level for the intact liver.

On the first days after orthostasis disturbances of the circulation in the small vessels and capillaries were observed in the organs studied, leading to both diffuse and localized degenerative changes. As a result the number of cells with karyolysis increased in the liver and adrenals and the number with pycnosis increased in the liver (Figs. 1 and 2). Meanwhile, the number of amitoses and binuclear cells increased in the liver also. By the third day an increase in mitotic activity of the hepatocytes was observed (MC = 0.49‰). On the fifth day the number of cells with karyolysis and pycnosis and also the number of binuclear hepatocytes in the liver were reduced. In the adrenal, a reduction in karyolysis was accompanied by an increase in the number of cells with pycnotic nuclei and the number of mitoses; the latter reached a peak on the seventh day (MC = 3.90‰). By the 10th-15th day the ratio between the numbers of cell forms in the adrenal was almost the same as initially, whereas in the liver at this time a second wave of amitoses appeared and the number of binuclear cells was increased. On the 20th day the number of hepatocytes with karyolysis and pycnosis of the nuclei was significantly higher than in the control ($P < 0.05$). The number of amitoses and binuclear cells at this stage was sharply reduced. As a result of these processes the cell composition of the liver in the later stages was relatively stabilized.

During recovery regular changes in the mean size of the cells also were observed in both organs (Fig. 3): a temporary increase in their dimensions (in the adrenal on the 1st and in the liver on the fifth day), evidently arising on account of swelling of the cells, and also a persistent decrease in the mean number of cells per field of vision of the microscope on the 30th-45th day of the experiment ($P < 0.05$), indicating their hypertrophy. The results of direct measurements of the cells and their nuclei are given in Table 1. In both organs there was an increase in the mean dimensions of the cells by 17-27% compared with the hemiadrenalectomized animals not exposed to orthostasis. In the liver, simultaneously with hypertrophy of the cytoplasm the mean area of the nucleus was increased ($P < 0.05$), whereas the nucleo-cytoplasmic ratio remained constant. The increase in the mean number of nucleoli per nucleus, with no change in their size, led to an increase in the nucleolo-nuclear ratio. The mean area of the nuclei in the adrenal was not increased but the nucleo-cytoplasmic ratio was reduced under these circumstances. Meanwhile the mean area of the nucleoli was increased by 300% without any corresponding increase in their number in the nucleus, so that there was a sharp rise in the nucleolo-nuclear ratio. The changes observed in the nucleolar system, characteristic of both organs studied, reflect an increase in the intensity of the rhythms of RNA synthesis in the nuclei [6], with a consequent increase in size of the cell cytoplasm.

The results point to an important role of hypertrophy of the cells, which evidently develops on account of intracellular repair processes, in the regeneration of the liver and adrenal cortex of rats after hemocirculatory lesions of these organs.

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