STIMULATION OF POSTTRAUMATIC REGENERATION OF THE RAT SPLEEN UNDER GRAVITATION OVERLOADING

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Posttraumatic regeneration of the rat spleen was studied after hemiresection of the organ under gravitation overloading (11 units) when spleen tissue extracts prepared by V. P. Filatov's formula were used as the stimulator. Under gravitation overloading the splenic nodules were smaller in size and smoother in outline, infiltration of their red pulp by lymphocytes was increased, the number of labeled cells and the intensity of label in the reactive centers of the splenic nodules were reduced, and capsule formation in the zone of resection was slowed down. As a result of the use of the stimulator the normal structure of the white pulp was restored, the number of labeled cells was increased, and capsule formation was more rapid. By using the tissue extract under gravitation overloading the process of regeneration (as regards the character and times of development of tissue differentiation) was brought more in line with the ordinary course of posttraumatic regeneration of the spleen.

KEY WORDS: gravitation overloading; regeneration of the spleen; splenic tissue extract.

Interest has recently increased in the investigation of the effect of extremal factors on animals and men. There is evidence in the literature on the significant effect of gravitation overloading on the histological structure of the intact spleen [1, 2], and on the veins and reticular tissue of that organ [3, 4]. However, no data on posttraumatic regeneration of organs under these conditions could be found. There are likewise no data on the use of stimulators under these conditions. The present writer has found that gravitation overloading adversely affects regeneration of the skin [5] and spleen [6].

The object of this investigation was to study posttraumatic regeneration of the spleen under gravitation overloading and during stimulation of the regeneration process.

EXPERIMENTAL METHOD

Experiments were carried out on 160 male albino rats weighing 130-150 g. Half of the spleen was removed from all the animals, which were distributed into three groups. Group 1 consisted of control rats; the animals of groups 2 and 3 were exposed to a gravitation overload of 11 units by spinning them on a special turntable in the head–pelvis position. Additionally, the rats of group 2 received a stimulator prepared from the spleen by V. P. Filatov's formula. The animals of all groups were decapitated 3, 7, 10, 20, and 30 days after removal of half of the spleen, six rats at each time. The numerical data were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

Under gravitation overloading appreciable changes took place in the pulp of the spleen. Whereas in the control animals moderate infiltration of the red pulp by lymphocytes took place, in rats exposed to gravitation overloading immediately after hemiresection of the spleen the red pulp contained more lymphocytes than erythrocytes.

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Under ordinary conditions of regeneration the number of splenic lymphatic nodules per field of vision was substantially unchanged, varying from 6 ± 0.23 to 8 ± 0.33. Under gravitation overloading, irrespective of stimulation their number at the same times of observation was reduced to between 4 ± 0.66 and 6 ± 0.49 per field of vision.

In all series of experiments the splenic lymphatic nodules in the zone of resection of the spleen in the early periods of observation (3-10 days after the operation) the boundaries between the splenic lymphatic nodules and the red pulp were indistinct. Multiple groups of lymphocytes, forming tiny islets, were observed in that region. However, the splenic nodules varied in size depending on the experimental conditions. For instance, whereas normally the diameter of the splenic nodules averaged 468.5 ± 0.89 μ, and in the control group by the 30th day of observation it was 374.6 ± 0.89 μ, under overloading conditions with or without stimulation their diameter did not exceed 340.7 ± 0.7 μ.

Besides counting the number of splenic nodules and measuring their diameter, we also counted the number of pyroninophilic cells in the reactive center. In relation to this index we subdivided the splenic nodules conventionally into three groups: those containing many pyroninophilic cells (up to 65 of the 300 cells in the field of vision), those with an average number – not more than 30, and nodules with only a few – not more than 15 pyroninophilic cells per field of vision. In the intact rats there were 45.2 ± 0.7% of group 1 nodules, 38.4 ± 0.1% group 2, and 16.4 ± 0.5% group 3. Changes in the number of pyroninophilic cells in the reactive centers of the splenic nodules are shown in Table 1. In the early stages in both the control and experimental animals the number of splenic nodules containing many pyroninophilic cells was reduced and, conversely, the number containing only a few such cells was increased. In the later periods of observation the opposite pattern was observed.

The results of counting the number of labeled cells after administration of thymidine-^{3}H and counting the number of reduced grains of silver per cell in the reactive centers of the splenic nodules were interesting (Table 2). Under ordinary conditions of posttraumatic regeneration, starting from the fifth day of observation,

TABLE 1. Distribution (in %) of Splenic Lymphatic Nodule by Number of Pyroninophilic Cells per Field of Vision (M ± m)

Group of animals	Splenic lymphatic nodules containing pyroninophilic cells, per field of vision, on undermentioned days of observation											
	7th day			10th day			20th day			30th day		
	not more than 65 per field of vision	not more than 30 per field of vision	not more than 15 per field of vision	not more than 65 per field of vision	not more than 30 per field of vision	not more than 15 per field of vision	not more than 65 per field of vision	not more than 30 per field of vision	not more than 15 per field of vision	not more than 65 per field of vision	not more than 30 per field of vision	not more than 15 per field of vision
Experimental, without stimulation	31,8±0,1	36,4±0,7	31,8±1,0	22,1±0,4	38,8±0,7	39,1±1,0	33,4±0,4	37,8±0,6	28,8±1,0	37,8±0,6	33,6±0,7	28,6±1,3
Control	25,2±1,0	31,2±0,9	43,6±0,8	24,8±1,1	33,2±0,9	42,2±0,9	38,6±1,2	34,6±1,4	26,8±1,0	39,8±1,1	34,0±0,9	26,2±0,7
Experimental, with stimulation	25,3±0,8	25,3±0,7	49,4±0,8	26,3±1,2	30,1±0,6	43,6±0,8	39,8±0,7	35,6±0,9	24,6±0,7	40,8±0,6	36,2±1,2	23,0±0,9

TABLE 2. Incorporation of Thymidine-^{3}H under Different Experimental Conditions (M ± m)

Group of animals	Time of observation							
	5th day		10th day		20th day		30th day	
	Number of labeled cells per 100	Number of grains per labeled cell	Number of labeled cells per 100	Number of grains per labeled cell	Number of labeled cells per 100	Number of grains per labeled cell	number of labeled cells per 100	number of grains per labeled cell
Experimental, without stimulation	21,3±0,5	10,1±0,6	13,5±1,0	10,3±0,8	17,1±0,9	29,6±0,8	30,3±1,2	30,1±0,5
Control	25,3±0,7	15,3±0,7	16,5±0,9	21,1±0,9	20,5±0,9	31,3±1,0	30,6±0,8	32,1±1,0
Experimental, with stimulation	21,3±0,9	12,1±0,7	18,5±0,7	13,1±0,7	20,5±1,1	31,0±1,0	33,1±0,8	32,1±1,3

the number of labeled cells was 25% and the number of grains of silver 15.3 per cell. Gravitation overloading reduced these figures to 21% and 10.1%, respectively. Under these conditions stimulation caused some increase in the number of labeled cells and in the number of grains of silver, and by the end of the observation (30th day) these indices corresponded to the control.

The effect of the experimental conditions was clearly manifested on capsule formation in the zone of resection.

By the 10th day of observation under ordinary conditions of regeneration proliferation of fibroblasts was observed in the region of resection. In some animals connective tissue cells were arranged horizontally in some places, but in many cases a thin connective tissue capsule was formed over the whole surface of the defect. In the experimental groups by the 10th day of observation there was marked delay in capsule formation. The zone of resection was covered by loose connective tissue. Cells were more numerous than connective tissue fibers.

On the 30th day after the operation the wound of the spleen in the animals of the control group was covered by a fully formed capsule. However, it was rather thinner than the capsule over the undamaged areas. In the animals exposed to overloading the wound surface of the spleen was covered by a thick layer of relatively undifferentiated tissue. Differentiation of the newly formed capsule was appreciably delayed compared with that of the control spleen. In the stimulated rats, by the 30th day after the operation the wound was covered by a capsule the structure of which closely resembled that of the control animals.

Repeated gravitation overloading after hemiresection of the spleen thus had a substantial effect on regeneration of that organ. The number and diameter of the splenic lymphatic nodules were reduced. The red pulp was abundantly infiltrated by lymphocytes. In the region of the wound there were many deformed lymphocytes. The number of pyroninophilic cells in the splenic nodules was reduced. The number of labeled cells and the number of grains of silver per cell were reduced.

When splenic tissue extract was used under these conditions as a stimulator the number of pyroninophilic and labeled cells and the number of grains of silver per cell were all increased, the structure of the pulp of the organ closely resembled that in the control, and the formation and differentiation of a capsule in the zone of resection took place more rapidly. On the whole, stimulation considerably abolished the phenomena due to gravitation overloading, so that the development of processes of regeneration in the resected spleen more closely resembled the ordinary course of posttraumatic regeneration of that organ.

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