Morphological Restructuring of Lymphoid Organs in Birds and Tailless Amphibians after Surgical Interventions on the Viscera

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Resection of the liver and pancreas in birds involves a decrease in mitotic activity of follicular lymphoid cells in the bursa of Fabricius (3.8 and 2.6 times on days 10 and 20 postoperation, respectively) and in the spleen (1.5-2 times 1-30 days postoperation) in comparison with the white pulp and splenic follicles volume. In frogs, unilateral nephrectomy involves devastation of the spleen (its red and white pulp), which does not decrease by day 8 postoperation.

Key Words: *regeneration; lymphoid tissue reaction; liver; pancreas*

An intervention on the majority of viscera on hemopoietic tissue is a strong stress causing a complex of similar changes in the histological structure of primary and secondary lymphoid organs [2-4,7,8].

The major manifestations of stress whose degree depends on the severity of stress and scope of intervention are involution of the thymus with a characteristic sharp decrease in its cell content, drastic and almost simultaneous decrease in cell content of the bone marrow with a simultaneous increase in the total count of lymphocytes and the appearance of T cells in it, and a short-term increase in the weight and cell content of the spleen followed by hypertrophy due to cellular hyperplasia [2,4,7,8].

Unlike sham operations, partial hepatectomy and unilateral nephrectomy involve an increase in lymphocyte counts in the periarterial zones as soon as several hours postoperation, which persists during the entire period of proliferation in the epithelium of regenerating liver and kidneys [3]. Increased cell content of the bone marrow is believed to be responsible for subsequent stimulation of myelo- and erythropoiesis [4,7,8]. Likewise, enrichment of splenic T-zones by lymphocytes stimulates proliferation [3]. The incidence of these events supports this hypothesis. We studied the reaction of lymphoid organs in general and of splenic T-zones in various periods after interventions on the liver, pancreas, and kidneys in birds and tailless amphibians characterized by slow re-generative processes [1]. Few reports offer but a general characterization of the spleen in doves after liver injury and in reptiles after tail injury and specify lymphoid cell hyperplasia in the white pulp of the spleen [11,12].

MATERIALS AND METHODS

Liver tissue (20% of distal part of the right lobe) and/or 16% pancreatic tissue were resected in white Lehgorn roosters (n=53) aged 2-2.5 months (group 1) and 5-6 months (group 2). This caused a potent stress similar to that observed in mammals after resection of 2/3 of liver tissue [3]; a more extensive intervention on each of these organ is impossible in birds [5,9,10]. Combined surgical trauma increased 7-fold an extremely low proliferative response of the

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liver to its resection during its maximum (day 3 postoperation) in group 2 [5,6].

In the frog *Rana temporaria* (n=12) one kidney was removed. This organ was chosen because its regeneration is best studied in tailless amphibians and because the results may be correlated to lymphoid tissue reaction in mammals [1,3]. Experiments were carried out in winter.

Roosters were decapitated 1, 3, 5, 10, and 20 days after intervention in group 1 and 1, 5, 10, 30, and 60 days postoperation in group 2.

Bursa of Fabricius, an analog of mammalian bone marrow, and the spleen were isolated, fixed in Bouine and Carnoi fluids, 5-μ paraffin sections were stained with hematoxylin and eosin, and impregnated with silver according to Foot. Frogs were decapitated 1, 2, and 8 days after unilateral nephrectomy [1]. Spleens were fixed in neutral formalin and 4-μ paraffin sections were stained with hematoxylin and eosin. Mitotic activity was assessed in follicles of the bursa of Fabricius, spleens (in birds), and renal canalicular epithelium (in frogs); 2000-3000 lymphoid cells and 4000 epithelial cells were counted and the mitotic activity was expressed in pro mille. The ratio of follicle, red and white pulp in the spleen was determined by the dot counting method.

Each group consisted of 3-6 animals. The resultant values were statistically processed using Fisher-Student's method.

TABLE 1. Mitotic Activity of Lymphocytes in Follicles of Bursa of Fabricius and Spleen in Roosters Postoperation ($M\pm m$)

Day postoperation	Mitotic activity, ‰	
	Fabricius' bursa follicles (group 1)	splenic follicles (group 2)
Control	3.6±0.92	27.01±0.94
1	5.5±1.55	47.45±4.45*
5	—	59.05±3.58*
10	13.8±1.02*	55.05±2.84*
20	9.3±1.79*	—
30	—	43.96±1.42*
60	—	38.41±5.43

Note. Here and in Table 2: *p<0.05 vs. control.

TABLE 2. Relative Volume of Splenic White Pulp and Follicles in Roosters after Partial Resection of the Liver and Pancreas ($M\pm m$)

Day postoperation	Relative volume, arb. U	
	white pulp	splenic follicles
Control	22.0±0.3	0.9±0.1
1	32.1±2.3*	2.7±0.7*
5	34.6±1.3*	3.8±0.6*
10	35.8±0.9*	3.5±0.4*
30	36.1±0.3*	4.5±0.5*
60	36.6±1.3*	2.9±0.3*

RESULTS

Histological analysis showed that the structure of the bursa of Fabricius changed 1 day after removal of a fragment of the liver. The number of cells in the cortical layer or medulla decreased in some follicles. However, in the majority of follicles the number of cells increased, while the proliferative activity was virtually unchanged. This increase is apparently similar to that in mammalian bone marrow, occurring at the expense of changed lymphocyte migration and their entry from the thymus, spleen, and peripheral blood. In later periods (days 10 and 20) the number of dividing cells increased 3.8 and 2.6 times respectively, in comparison with the control (Table 1).

In birds, the reaction of the spleen to resection of the liver and pancreas was virtually the same as in mice, but some of its stages were stretched in time. On day 1 postoperation, devastation of the white and even more so of the red pulp was observed; as a result, the volume of the white pulp increased (Table 2). T zones rich in lymphocytes were practically not observed during this period or were detected as a narrow periarterial rim. The volume of its bursa-dependent nodules was enlarged, but their cell content was decreased in comparison with the norm and other periods of observation (Table 2).

The relative volume of white pulp remained increased in comparison with the norm up to day 60 postoperation, which is in line with previous findings [3], and virtually did not change till the end of the observation period, as well as the volume of bursa-dependent nodules (Table 2). The level of mitotic activity of their lymphoid cells remained increased up to day 30 postoperation, being the highest on days 5 and 10 postoperation (Table 1). T zone increased starting from day 5 postoperation but still looked as a discernible periarterial rim. The T zone was the largest by day 10 (Fig. 1) and decreased again on days 30 and 60 after intervention. In roosters, unlike in mammalians, proliferative activity of hepatocytes in regenerating liver was increased on day 10; complete stable regeneration of the liver was observed only on day 30 after the injury (but not on days 7-10, as in mice) [6,10].

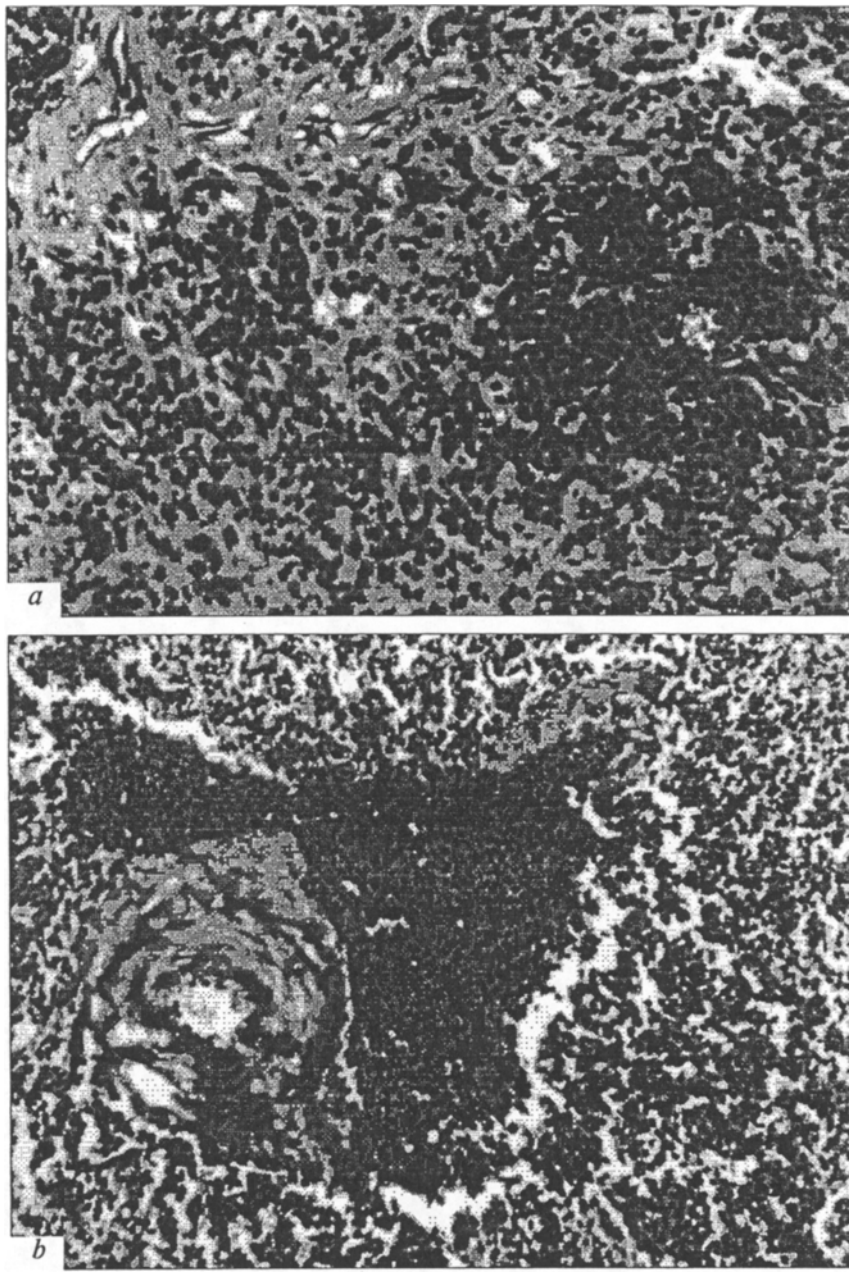

Fig. 1. Rooster spleen. *a*) follicle and periarterial zone in control; *b*) increased cellular content of periarterial zone on day 10 postoperation. Hematoxylin-Eosin staining, ×200.

In frogs, despite decreased vital activity in winter, unilateral nephrectomy caused progressing devastation of the red and white pulps of the spleen, which showed no tendency to regeneration on day 8 postoperation. No lymphocyte concentrations were detected in T zone of operated animals at any period. No mitoses were observed in the kidneys of control and operated animals. In general, changes in splenic histostructure of unilaterally nephrectomized mice indicated a pronounced stress reaction, which was similar to that in unilaterally nephrectomized mice during the first hours after intervention [3]. This period was longer in frogs than in birds or mice, as was the duration of regeneration of the bulk of damaged organs [1].

The rate of organ repair in different vertebrates depends on the rate of manifestation of lymphoid organ stress reaction. The lag-period of stress reaction is the longest in tailless amphibians, shorter in birds, and the shortest in mammals. Long duration of some periods of postoperative stress and low proliferative activity of cells in regenerating organs in birds and tailless amphibians did not allow us to detect its relationship with the state of T zones, like in mammals [3].

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