Structure and Function of the Thymus during Adaptation of Rats to Hypergravitation

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 2, pp. 219-222, February, 2003
Original article submitted September 9, 2002

Five-day exposure to hypergravitation (2*g*) induced structural and functional changes in the thymus: intensification of cell destruction and inhibition of mitotic activity of lymphocytes in all functional components of the organ. This leads to a significant (2-fold) decrease in thymus weight, area of the thymus cortex on histological section, blurring of the corticomedullary interface because of lymphocyte depletion of the cortical matter. The size and number of thymic bodies decrease 3.4 times compared to the control. This indicates that by day 5 of continuous hypergravitation (2*g*) exposure the lymphoid tissue does not completely adapt to hypergravitation.

Key Words: *thymus; lymphocyte; hypergravitation*

Search for approaches and methods for prevention of unfavorable effects of space mission factors, such as hypokinesia, microgravitation, *etc.*, on humans remains one of the most important tasks of space biology and medicine. The use of artificial gravitation is considered to be the most perspective method for such studies [2,4]. Structural and functional changes in organs and tissues during long-term exposure to hypergravitation of moderate intensity are now actively studied by space biology [6,7]. However, these studies pay little attention to investigation of the immune system, which plays an important role in the maintenance of homeostasis and stability of its antigenic structures and in regulation of genetic stability of somatic cells [9].

We investigated the structure and cytoarchitectonics of rat thymus during prolonged exposure to hypergravitation of 2*g*.

MATERIALS AND METHODS

Adult male Wistar rats (n=20, 10 per group) were used in the study. Hypergravitation was simulated by rotation of animals in peripheral cages of a CFKB-365 centrifuge (radius 1.41 m) at 33.3 rpm. Gravitation increment and decrement coefficient was 0.02 *g*/sec. The animals were not fixed during rotation. The rotation was permanent, with only 20-min daily stop for cleaning the cages and giving the fodder. The duration of exposure was 5 days. According to published reports, at this term the intensity of nonspecific changes caused by gravitation stress decreases, while specific adaptation changes become more pronounced [5,6]. After the end of the experiment the animals were decapitated. The thymus was fixed in 10% neutral formalin, treated with ethanol, and embedded in paraffin. Histological sections (4-6 μ) were stained with hematoxylin and eosin and azur II-eosin. The areas of thymic structures were estimated on histological sections as described previously [1] and the cortico-medullary index was calculated. The number of cells per unit of arbitrary area (880 μ^2) of histological section was counted and their percent ratio estimated in order to study the cytoarchitectonics of the structural and functional components of the thymus. The significance of differences was evaluated using Student's t test.

RESULTS

Continuous 5-day exposure to 2*g* hypergravitation resulted in an almost 2-fold decrease in thymus weight compared to the control. In the thymus of experimen-

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0007-4888/03/1352-0190$25.00

tal rats, the cortex area on histological sections decreased 1.3 times (Table 1), while the area of the medulla increased 1.2 times compared to the control; the number of thymic bodies in the thymus lobule decreased 3.4 times; the corticomedullary interface was blurred (Fig. 1). Published data suggest that hypergravitation induces hemodynamic disorders in organs and tissues [5]. In light of this the reaction of the microcirculatory bed of the thymus in experimental animals is of particular importance. We detected some differences in the reaction of the arterial and venous systems of the thymus to hypergravitation. Arterial walls were thickened because of loosening and edema after 5-day continuous exposure to 2*g* hypergravitation. Perivascular zones were edematous. Arteries were primarily empty and collapsed. By contrast, veins were dilated and plethoric, destruction of venous walls and release of blood elements into the thymic parenchyma were sometimes seem. Erythrocyte aggregation was seen in the capillary lumen. Small hemorrhagic foci (primarily diapedesis) were detected in the thymus parenchyma. Disorders in the circulatory bed lead to edema and loosening of the thymus capsule and interlobular connective tissue in rats after 5-day continuous hypergravitation.

Analysis of cell composition in structural and functional components of the thymus revealed pronounced changes at the cellular level after 5-day continuous exposure to hypergravitation (Table 2). The cell density in the subcapsular zone decreased 1.4 times in comparison with the control (Table 2). This was mainly due to decreased number of blast forms and minor lymphocytes. The percentage of large and medium lymphocytes little changed. In parallel, the mitotic activity of lymphocytes in this zone decreased 1.3 times. Cell destruction processes in the thymus were intensified in experimental animals and macrophage count decreased 1.2 times compared to the control. The number of plasma cells in the subcapsular cortex increased 5-fold after hypergravitation exposure.

Similar changes were observed in deep layers of the thymic cortex after 5-day hypergravitation; but in these zones the most pronounced decrease was observed in the number of minor lymphocytes (1.3 times).

TABLE 1. Percent Ratios of Areas of Structural Components of Rat Thymus in the Control and after Exposure to 2*g* Hypergravitation ($X \pm Sx$)

Structure	Groups	
	control	experiment
Capsule	17.28±1.33	24.27±1.20*
Cortex	40.88±4.79	31.98±1.58***
Medulla	29.34±3.95	35.31±1.35***
Thymic bodies	4.99±0.61	2.86±0.56***
Cortical vessels	4.80±0.56	3.73±0.72
Medullary vessels	2.71±0.31	1.84±0.36***
C/M index	3.44±0.58	0.94±0.07*

Note. Here and in Table 2: *p<0.001, **p<0.01, ***p<0.05 compared to the control.

TABLE 2. Percentage of Cells in Structural Components of Rat Thymus in the Control and after Exposure to 2*g* Hypergravitation ($X \pm Sx$)

Cells	Cortex				Medulla	
	subcapsular zone		deep zone			
	control	experiment	control	experiment	control	experiment
Blasts	5.82±1.27	2.43±0.86***	2.91±0.64	2.58±0.82	1.46±0.61	—
Large lymphocytes	8.49±1.30	6.85±1.71	6.88±0.99	7.36±1.12	6.91±1.12	4.12±1.90
Medium lymphocytes	19.43±1.32	20.87±2.20	17.89±2.56	22.70±2.37	30.23±3.07	15.04±2.72*
Minor lymphocytes	54.29±2.79	44.06±2.20**	67.94±2.69	51.29±2.25*	45.89±3.13	41.68±4.00
Dividing (mitotic)	1.45±0.45	1.14±0.60	1.51±0.45	—	0.23±0.22	—
Plasmatic	0.19±0.18	0.95±0.50*	—	0.22±0.21	—	—
Eosinophils	0.73±0.24	—	—	—	—	—
Neutrophils	0.17±0.16	—	—	—	—	—
Epithelioreticulocytes	7.75±1.27	21.13±2.28*	1.80±0.41	13.41±1.50*	10.39±1.65	33.61±249*
Macrophages	0.34±0.23	0.28±0.27	—	—	0.77±0.40	1.41±0.78
Destroyed	1.35±0.58	2.28±0.51	1.08±0.35	2.44±0.52*	4.13±1.15	4.15±1.26
Cell density	39.60±1.01	28.33±0.13*	37.20±1.40	30.33±0.62*	27.80±0.95	21.73±1.04*

Note. Cell density: absolute content of cells per arbitrary area unit on histological section (880 μ^2).

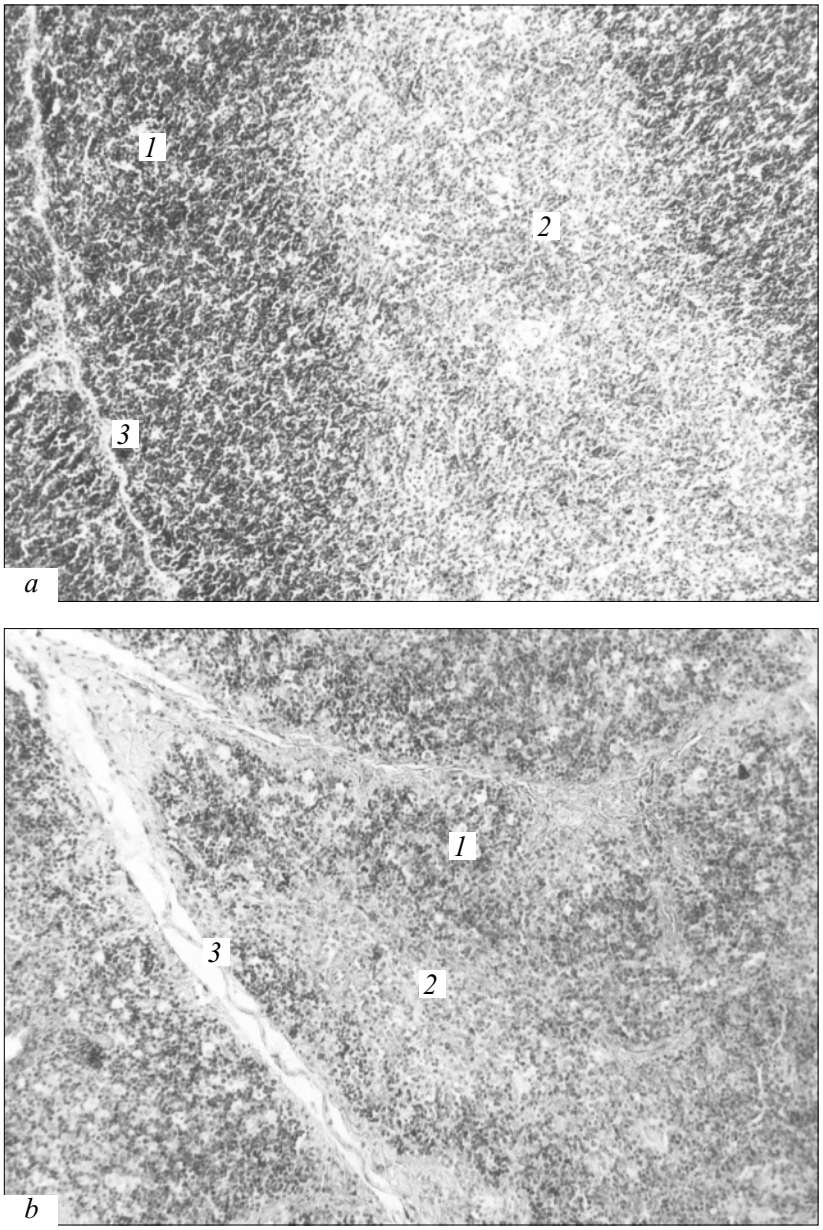

Fig. 1. Histological structure of the thymus in Wistar rats. *a*) wide cortical layer and clearly seen corticomedullary interface in the thymus of a control rat; *b*) narrow cortical layer, blurred corticomedullary interface, loosened and thickened interlobular septum in the thymus of a rat exposed to continuous 5-day 2*g* hypergravitation. Hematoxylin and eosin staining, ×100. *1*) cortex; *2*) medulla; *3*) interlobular septum.

Activation of cell destruction processes (by 2 times) and inhibition of cell proliferation were noted. There were virtually no dividing lymphocytes in deep layers of the thymic cortex of experimental rats. All this led to emptying of the cortex, which was seen from a significant decrease in the cell density per unit of the histological section area in this zone ($p<0.001$).On the other hand, the total count of large and blast lymphocytes in the cortex remained practically unchanged. The number of medium-sized lymphocytes increased 1.3 times, which probably attests to activation of lymphocyte differentiation (Table 2).

Hypergravitation produced similar effects on the functional activity of the medulla (Table 2). Since the medulla is the site of final differentiation of T lymphocytes, the most important morphological parameter of this structure is the content of medium-sized and minor lymphocytes. The number of minor lymphocytes in the medulla of experimental animals little differed from the control. The content of medium-sized lymphocytes decreased 2-fold (hypergravitation inhibits lymphocytopoiesis both in the cortex and medulla). The total content of blasts and large lymphocytes in the thymic medulla of experimental rats decreased 2-fold in comparison with the control; no cells in mitosis were seen. The percentage of destroyed cells in the medulla slightly decreased, which was compensated by increased (2-fold) number of macrophages.

Our findings are in line with previous data [3] on decreased volume of lymphoid tissue in the spleen of rats mainly due to a sharp decrease in the size and number of germinal centers in the lymphoid follicles

after similar exposure. Moreover, the number of dividing cells at different stages of mitosis in lymphoid follicles decreased, which attested to impaired splenic lymphocytopoietic function. The thymus weight decreased, while the weight of the adrenals increased in the same rats [6]. This was paralleled by an increase in blood eosinophil count. It was shown that 5-day 2*g* hypergravitation stimulated proliferation of neutrophilic granulocytes and decreased the number of lymphoid cells in the bone marrow [6].

Hence, continuous 5-day exposure to 2*g* hypergravitation suppressed lymphocytopoiesis and enhanced destructive processes in all structural and functional components of the thymus. These results indicate that no complete adaptation of lymphoid tissue to hypergravitation is attained by day 5 of hypergravitation (2*g*) exposure.

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