

## MORPHOLOGY AND PATHOMORPHOLOGY

# Effect of Physical Loads of Different Intensity on Elements of the Lymphoid Tissue of the Thymus and Spleen

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As has been established, severe physical stress of any nature compromises the organism's immune status [9,11], culminating in the development of autoimmune and allergic disorders [3] and immunodepression [5]. There are only a few publications testifying to disorders in the structural organization of the organs involved in immunogenesis which go along with an increase [2,7] or decrease [6] of motor activity. Main attention is paid to the predominant component of the lymphoid tissue, namely the lymphocytes. On the other hand, the cells that are at the early stages of differentiation, namely, competent-for-mitosis blastocytes and large lymphocytes, have been poorly studied.

Since the role of these cells in the system of immunobiological control is of great importance [1,4, 12], we studied the changes in the content of blastocytes and large lymphocytes under physical loads of different intensity or under immobilization stress.

### MATERIALS AND METHODS

The experiments were performed on 6-8-month-old female BALB/c mice. For modeling severe physi-

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cal load, 6 mice were made to swim at a water temperature of 38°C during 2 weeks (4 days a week, 3 times a day for 20 min). In the modeling of moderate physical load, the conditions were the following: swimming during 2 weeks, 4 days a week, 3 times a day for 6 min at 28°C and 6 min at 38°C. When immobilization stress was modeled, 6 mice were placed in plastic tubes for 6 h a day with access to food and water ad libitum. Six control mice were kept under the same conditions. Structural components of the thymus and spleen were studied by biomorphometry in histological preparations obtained from 18 animals. The numerical data (absolute and relative % indexes) were subjected to statistical analysis. Paraffin sections 4-5  $\mu$  thick were stained with Azure II-eosin after Brachet. The tissue samples were fixed in 10% buffered formalin after Lillie. The absolute number of blastocytes, large lymphocytes, and cells with pictures of mitosis in the structures of the thymus (subcapsular zone, cortex, and medulla) and spleen (central and peripheral zones of the lymph node and the red pulp) per unit area (880  $\mu^2$ ) was determined in all experimental groups and compared with the analogous indexes in the control.

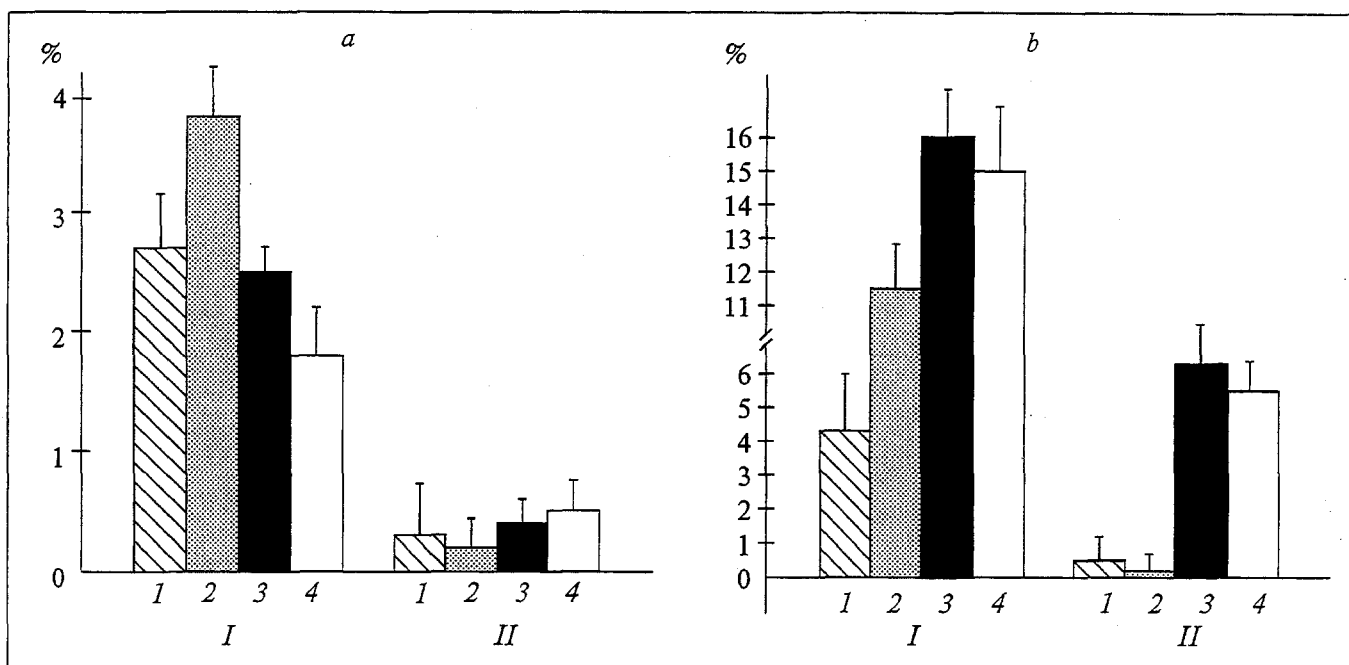


Fig. 1. Content (in %) of dividing (a) and poorly differentiated (b) cells in the thymus and spleen under physical loads of different intensity. I) subcapsular zone of thymus, II) central zone of splenic lymph node. Abscissa: group of animals; ordinate: cell content (in %). 1) control; 2) intensive swimming; 3) moderate physical load, 4) immobilization stress.

## RESULTS

Analysis of cells in different regions of the spleen showed that in experimental animals under immobilization stress the absolute number of large lymphocytes in the central zone of the lymph node increases 9-fold in comparison with the control, 21-fold in the mantle, and 11-fold in the red pulp sinuses. Blastocytes were not found in the spleen of the control animals. In the experimental animals the highest content of these cells was recorded in the center of the lymph node ( $1.18 \pm 0.37\%$ ), whereas in the mantle zone and the red pulp blastocytes were absent (insignificant differences with the control). In the spleen of experimental animals cells with mitotic figures first appeared in the mantle ( $0.312 \pm 0.09\%$ ) and occurred twice as frequently in the center of the lymph node compared to the control. Mitotic activity was enhanced in the red pulp ( $0.81 \pm 0.30\%$  compared to the control  $0.03 \pm 0.03\%$ ). These findings testify to activation of the reproductive function of the lymphoid tissue of the spleen in response to immobilization stress.

Under moderate and intensive physical loads the content of the cells under study in the splenic structures was almost the same, except for the large lymphocytes, which were more abundant in the mantle zone under a moderate physical load.

As for the thymic structures, shifts in the content of blastocytes, large lymphocytes, and cells

with mitotic figures had mosaic pictures in different experimental groups. In the most reproductive, subcapsular, zone of the thymus, the number of blastocytes increased most of all under the moderate physical load (4-fold in comparison with the control) and to a somewhat lesser extent for intensive swimming. In the thymic cortex, the increase in the number of blastocytes was most pronounced during intensive swimming (7-fold in comparison with the control). Upon the moderate physical load and immobilization stress, the concentration of these cells was 50% lower but still 3-4 times higher than the control level. In the medulla of the thymus, the content of blastocytes was the lowest and this structural component practically did not react to the physical load (insignificant differences in comparison with the control).

As for the large lymphocytes, it is noteworthy that the accumulation of these cells proceeded most actively in the thymic medulla. Under immobilization stress, the share of large lymphocytes increased 20 times ( $1.85 \pm 0.76\%$  in the experiment and  $0.09 \pm 0.03\%$  in the control). Under intensive and moderate physical loads the share of these cells constituted  $0.86 \pm 0.03\%$  and  $1.00 \pm 0.39\%$ , respectively. The highest content of large lymphocytes was recorded in the subcapsular zone of the thymus, and their number significantly increased in all types of experiments performed (Fig. 1). The accumulation of large lymphocytes is most inten-

sive in the thymic cortex during the moderate physical load, when the number of these cells per unit area ( $880 \mu^2$ ) increases 3.5 times, while under immobilization stress or intensive swimming this index becomes approximately twice as high in comparison with the control.

The dynamics in the number of cells with mitotic pictures in the thymus was most interesting. In the thymic medulla in the experimental animals, the changes in the content of these cells correlated with the content of the cells at early stages of differentiation. In the subcapsular zone and cortex of the thymus, under immobilization stress or during the moderate physical load the level of these cells dropped 2-fold in comparison with the control. Only under a severe physical load such as intensive swimming at  $38^\circ\text{C}$  did we record activation of mitosis in all structural components of the thymus. This finding may be regarded as evidence of the adaptive enhancement of mitotic activity in the thymus, which extends its reproductive function even as far as the deep layers of the cortex.

Generalization of the experimental data made it possible to reveal the following regularity in the dynamics of the total number of cells under study in the two organs. In the spleen the most pronounced changes occur under immobilization stress, i.e., when mobility is restricted: the total number of cells at early stages of differentiation (large lymphocytes and blastocytes) and of cells in a state of mitosis increases, respectively, more than 14 and 10-fold in comparison with the control. Moderate and severe physical stress leads to less pronounced changes in these indexes, although their absolute values exceed the control level. In the thymus, during intensive physical exercises, the number of poorly differentiated and dividing cells (at the stage of mitosis) increases 2.5 and 1.4 times, respectively, in comparison with the control. Under moderate physical load, the first index increases 2.7-fold, whereas the second one becomes 50% lower than in the control. Under immobilization stress, the total number of poorly differentiated cells in the thymus increases 2.4-fold, whereas the number of dividing cells decreases 2.4-fold. We assume that these shifts may testify to inhibition of cell reproduction in the thymus under immobilization stress and during moderate physical exercise. It is most likely that the low level of T lymphocytes in blood of athletes [8,10] and reduc-

tion of the proliferative activity [12] of these cells are due to their inhibited reproductive function. Under a severe physical load, the changes of cell composition in the thymus are less pronounced in comparison with other types of physical influence.

As for the spleen, the most pronounced shifts towards an increase in the cell number are recorded during immobilization stress. This may be connected with the role of the spleen in the genetic control over elements of the blood.

Thus, the experiments performed have shown that severe physical stress leads to enhanced mitotic activity in all thymic structures. In the spleen, on the other hand, this shift occurs when the animals' mobility is restricted, and it is accompanied by intensive accumulation of blastocytes and large lymphocytes. In the thymus, these indexes are dependent on both the type of physical influence and the structural component of the organ. The second peculiarity of the thymus is an increase in the share of large lymphocytes and blastocytes in all structures. In the spleen the content of these cells is reduced in the peripheral zone of the lymph node under a severe physical load and in the red pulp under a moderate load. The observed changes in the thymus and spleen under physical loads of different intensity may be attributed to the formation and transformation of the lymphoid cells and, probably, to their migration within and outside of the structural elements of these organs.

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