

EXPERIMENTAL BIOLOGY

Reaction of Thymic Monoamine-Containing Structures to Experimental Testectomy

V. E. Sergeeva and I. L. Sarilova

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Testectomy increased serotonin content in fluorescent granular macrophages of the subcapsular and premedullary zones and in mast cell granules. Serotonin concentrations in the medullary and cortical thymocytes decrease. Young sudanophilic mast cells with low content of proteoglycans and low-sulfated immature heparin predominate in the gland after testectomy.

Key Words: *testectomy; thymus; subcapsular and medullary zone macrophages; mast cells*

There are different opinions on the effects of androgens on the immune processes. Injection of testosterone *in vivo* and its addition to cell cultures suppress humoral immune response. Cellular immune response to exogenous sex hormones is also suppressed, which is paralleled by suppressed development of the thymus and peripheral lymphoid tissue and induction of T-suppressors in lymphocyte population [3].

Stimulation of the immune response after experimental sex hormone insufficiency (castration) was observed at the expense of increase in T-cell count [3]. Presumably, the hormone modifies activity of bone marrow polypotent stem cells, stimulates proliferation and migration of these cells, and promotes their differentiation towards erythropoiesis pathway to the detriment of lymphopoiesis, which probably underlies the immunosuppressive effect of testosterone. Along with glucocorticoid, estrogen, and progesterone receptors, thymocytes express receptors for androgens and neurotransmit-

ters, which confirm close relationship between the immune, nervous, and endocrine systems [6,8,9].

We studied the structure of the population of bioamine-containing cells of the thymus, the central immune organ, in male sex hormone insufficiency after experimental testectomy (TEC).

MATERIALS AND METHODS

The thymus of 24 outbred male rats (150-200 g) was studied. Bioamine-containing thymic structures of thymectomized rats were studied in autumn. This season was chosen as the most stable for the content of biogenic amines in thymic structures. The animals were divided into 2 groups: control ($n=12$, intact) and experimental ($n=12$, TEC). TEC was carried out with due consideration for all aseptic and antiseptic regulations. All manipulations on animals were carried out in accordance with the standards and regulations on handling laboratory animals. The thymus was removed under deep ether narcosis on day 30 after castration. Cryostat sections were processed by the histochemical method [7] for detecting thymic structures containing serotonin (5-HT) and catecholamines and examined under

Department of Medical Biology, I. N. Ul'yanov Chuvash State University, Cheboksary

a LUMAM-4 fluorescent microscope at $\lambda_{\text{ex}}=360$ nm. Spectrofluorometry was applied for identification and measurements of 5-HT and catecholamines in thymic structures. 5-HT was identified using $\lambda=525$ photofilter, catecholamines with $\lambda=480$ photofilter. The values were recorded from the amplifier in arbitrary units. In order to clear out the effect of monoamine, playing a leading role in the thymus after TEC, 5-HT index ($I_{5\text{-HT}}$) was estimated by the formula:

$$I_{5\text{-HT}} = \Sigma(5\text{-HT}_c / \text{CA}_c) / n$$

where 5-HT_c is 5-HT concentration in one cell, CA_c catecholamine concentration in one cell, and n is the number of cells.

If $I_{5\text{-HT}} > 1$, 5-HT predominates in the cell, if $I_{5\text{-HT}} < 1$, catecholamines predominate.

The status of mast cell granules was evaluated by heparin maturity using polychromatic toluidine blue staining after Unna. The following cell variants are classified by mucopolysaccharide state: α -orthochromatic mast cells (MC) with blue-stained cytoplasmic granules containing nonsulfated immature heparin; β_1 -metachromatic MC with violet-stained granules in the cytoplasm and more sulfated immature heparin; β_2 -forms with violet granules in the cytoplasm, with a reddish shade due to maturation of sulfated heparin; β_3 -metachromatic MC with red-violet cytoplasm and almost mature heparin; purple γ -metachromatic MC with completely sulfated mature heparin in granules.

The following MC forms are distinguished in the thymus by degranulation degree [4]: T_0 -forms, with granules compactly packed in the cytoplasm and indiscernible nucleus; T_1 -forms, with well seen nucleus, granules inside the cells, but not outside

the cytoplasmic membrane; T_2 -forms, with granules partially outside intact cytoplasmic membrane; T_4 -forms - completely degranulated MC with disrupted cytoplasmic membrane. Sudan Black B staining was used for detecting intracellular lipids (bioamine binding substrate). Hematoxylin and eosin staining was used as common histological staining.

RESULTS

Hypertrophic thymus with enlarged lobules was usually seen in castrated rats. Lobular medulla was extended. Inversion of the medulla towards the cortical matter was seen in some lobules of the gland. Premedullary macrophages fluorescing bright yellow and yellowish-green formed one or two rows in the inner part of the lobular cortical layer in the corticomedullary zone. Fluorescent granular cells of the subcapsular zone chaotically scattered at the periphery of the lobular cortical matter fluoresced yellowish-green. Sections of the thymus of castrated animals showed, along with large lobules, small accumulations of fluorescent granular cells forming open semi-rings, with one row of cells. Presumably, these formations are newly forming lobules. Mast cells were detected along blood vessels in interlobular septae, at the interface between the cortical matter and medulla, and among thymocytes of the lobular cortical matter. The cells had dark nuclei in the center and bright yellow fluorescent granules in the cytoplasm.

The content of 5-HT sharply increased in amine-containing macrophages of the corticomedullary and subcapsular zones of the thymic lobules and in MC of castrated animals (Fig. 1, *a*). The intensity of 5-HT fluorescence in medullary and cortical thymocytes decreased after TEC. Fluorometric analysis

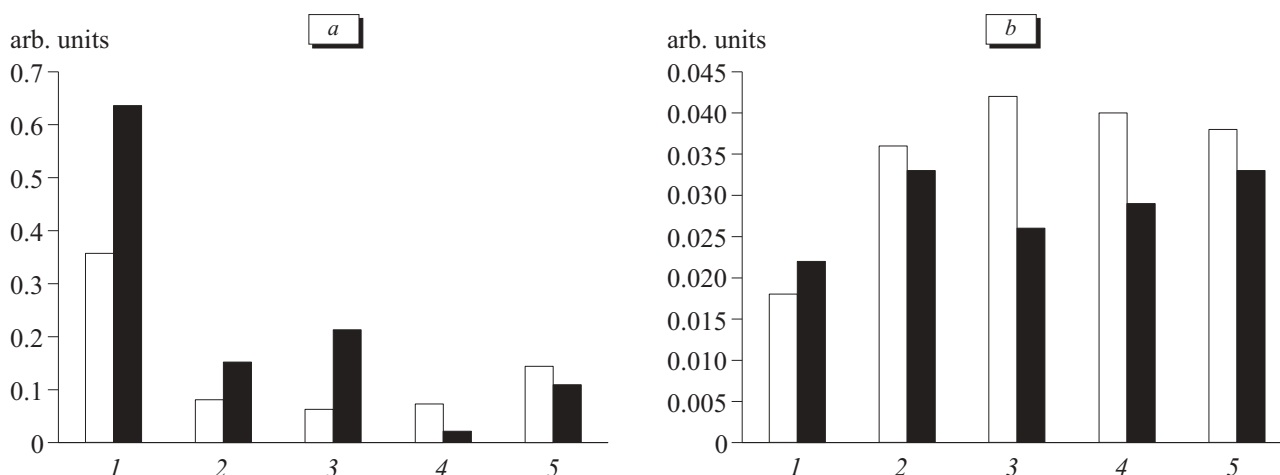


Fig. 1. 5-HT (*a*) and catecholamine (*b*) content in thymic microstructures after testectomy. 1) premedullary macrophages; 2) subcapsular macrophages; 3) mast cells; 4) medullary thymocytes; 5) cortical thymocytes. Light bars: control; dark bars: testectomy.

of catecholamine content revealed an increase in the level of this amine only in premedullary macrophages and its decreased concentration in other bioamine-containing structures of the thymus (Fig. 1, *b*). I_{5-HT} of fluorescent granular cells in the premedullary and subcapsular zones and of MC notably increased, while I_{5-HT} of cortical and medullary lymphocytes decreased after castration in comparison with the same structures in intact animals.

The population of β_2 -metachromatic MC with numerous red-violet granules (due to sulfated heparin maturation) predominated in intact male rats. The granules of these cells were partially released beyond the intact cytomembrane (T_2 -forms). After TEC β_1 -metachromatic MC with violet granules in the cytoplasm predominated in the thymic capsule and interlobular septae, indicating that they contained poorly sulfated immature heparin. The granules in these MC were located inside the cell, were well seen, and were not released outside the cytoplasmic membrane (T_1 -form); the number of young MC forms increased and the count of degranulated cells decreased significantly in the gland. Sudan Black B staining with prolonged hydrolysis showed sudanophilic MC with grayish granules. The cytomembrane of some cells was destroyed. Mast cells with small round black-blue granules were detected in the lobular parenchyma after TEC.

The macrophagic nature of fluorescent granular cells of the thymus is proven [1,2,5]. Initiation of the immune response, particularly T-cell mediated response, largely depends on thymic macrophages. A thymocyte with high 5-HT content can be qualified as a suppressor cell. The drop of 5-HT level in fluorescent granular cells can be determined by transfer of this bioamine to thymocytes. After TEC the concentration of 5-HT sharply increased in premedullary and subcapsular macrophages

and in MC. This was paralleled by a decrease in the content of this bioamine in medullary and cortical thymocytes. The decrease in I_{5-HT} index of cortical and medullary thymocytes after TEC can be regarded as 5-HT release from these structures, while the increase in I_{5-HT} of premedullary and subcapsular macrophages indicates accumulation of 5-HT.

Hence, deposition of suppressor bioamine 5-HT in fluorescent granular macrophages of the premedullary and subcapsular zones and in MC after TEC creates favorable conditions for T cell maturation, which manifests in enlargement of the lobules, hypertrophy of the thymus, and formation of new lobules. The predominance of MC with sudanophilic granules containing poorly sulfated heparin indicates the increase in the count of young MC against the background of appreciable reduction in the count of degranulated forms.

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