SEASONAL CHANGES IN AMINE-CONTAINING STRUCTURES OF THE THYMUS WITHIN 1 HOUR AFTER ANTIGENIC STIMULATION

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Significant seasonal changes in concentrations of serotonin (5-HT), noradrenalin (NA), and histamine (HA) have been discovered in the brain [2, 4, 5, 7, 8], the walls of blood vessels [6], rat peritoneal tissue basophils [1], spleen [10], and thymus [9].

The aim of this investigation was to study quantitatively and qualitatively seasonal changes in monoamine-producing microstructures of the thymus (neural and nonneural) during the first hour of contact of an animal with corpuscular antigen.

EXPERIMENTAL METHOD

Experiments were carried out on 60 Wistar-2 rats and on laboratory albino rats. Evaluation of the experimental results took into account the animals' age, sex, and body weight, the time of year, and the time of day. The model of antigenic stimulation consisted of intravenous injection of heterologous (sheep's) red blood cells (SRBC) in a dose of $7 \cdot 10^8$ cells in 1 ml physiological saline. Animals receiving an injection of 1 ml physiological saline with ink (10:1) and intact rats served as the control. The thymus was taken for study 15 and 60 min after injection in the experimental and control groups. The experiments were carried out in spring (May-June) and In the fall (September, October, and November) The following methods were used 1. The Falck–Hillarp luminescence-histochemical method was used as the basic and definitive method [11] in Krokhina's [3] modification for selective demonstration of adrenergic nerve fibers and also of the bioamine-containing structures of the thymus. Preparations were examined under the LYUMAM-1A microscope with excitation wavelength of 360-410 nm. Catecholamines were detected with the aid of a No. 6 interference filter (480 ± 10 nm), serotonin with a No. 8 filter (525 ± 11 nm). 2. Microspectrofluorometry for identification and quantitative estimation of NA and 5-HT levels was carried out with the same interference filters. The statistical significance of the results was determined by Student's test and the nonparametric Wilcoxon–Mann–Whitney test. 3. The Masson–Fontana method for histochemical identification of indole-containing structures of the gland. 4. Staining with Sudan black B after prolonged hydrolysis was used to detect lipid complexes – the substrate for bioamine binding. 5. Staining with alcian blue and sairanine was used to determine glucosaminoglycans In the tissue structures. 6. Staining with Unna's toluidine blue to detect the carbohydrate component of mast cells. 7. Glenner's method was used to determine MAO enzyme activity. 8. General survey staining with hematoxylin and eosin.

EXPERIMENTAL RESULTS

The microspectrofluorometric study of catecholamine (CA) and 5-HT levels in structures of the intact thymus in Wistar-2 and laboratory albino rats revealed different levels of bioamine production at different times of year (Figs. 1 and 2).

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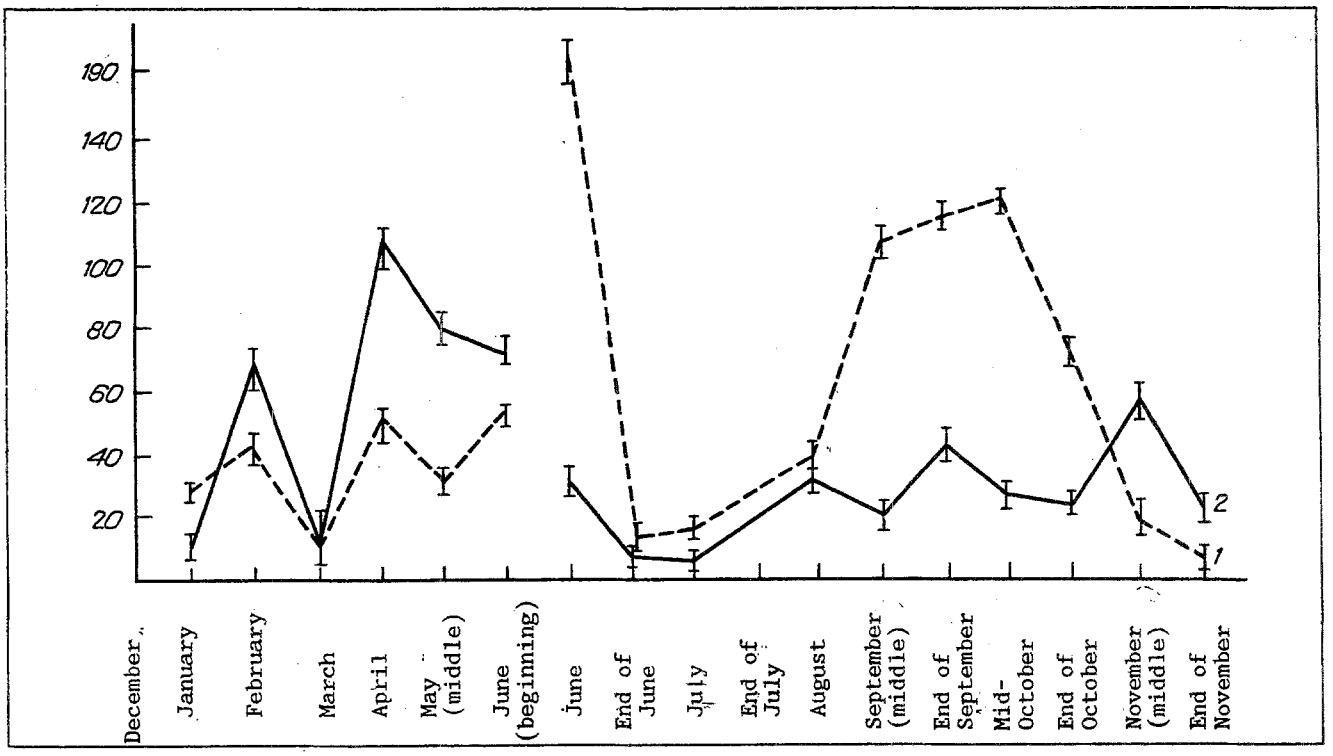

Fig. 1. Time course of intensity of fluorescence of catecholamines (1) and serotonin (2) in adrenergic nerves of intact rat thymus (conventional units). Abscissa, months of investigation; ordinate, mean intensity of fluorescence of bioamines.

In spring (May-June) the content of monoamines in luminescent cells of the premedullary and subcapsular zones, in mast cells, and in adrenergic nerve fibers was quite high. The density of the sympathetic innervation was increased and the nerves appeared thick and bandlike (Fig. 3a). Sudanophilic thymocytes and mast cells predominated in the thymus. Amine-containing cells were arranged in two rows in the cortico-medullary zone (Fig. 3c); their reaction for MAO was weak, and their glycosaminoglycan content reduced. Bright whitish-yellow mast cells with a high monoamine content were always discovered in the septa. According to the experimental data, in September the serotonin level in the monoamine-containing structures of the thymus was almost unchanged (Fig. 2). Meanwhile, the intensity of luminescence of CA in macrophagelike cells of the premedullary and subcapsular zones and also in the mast cells was observed to be reduced. A similar tendency also was found in the adrenergic nerve terminals. The process of lowering of the bioamine level continued in October and November in sympathetic nerves. Although no significant changes in the intensity of luminescence of these monoamines was found In the mast cells in October and the first half of November, nevertheless small granules with a reduced concentration of 5-HT and CA were discovered in the cytoplasm of these cells. This decrease may perhaps be connected either with reduced 5-HT production by the tissue basophils themselves or with intensified synthesis of heparin proteoglycan in their amine-inactivating system. Increased heparin synthesis in the mast cells during hibernation was found in the thymus, when alcianophilic cells predominated in the gland. Similar observations were made in [8]. A fall of the bioamine level was observed in the thymocytic parenchyma of the medulla and cortex of the thymic lobule.

In the fall, on the other hand, when there was a general decline in the neurotransmitter levels in the above-mentioned structures of the thymus, injection of heterologous antigen caused a significant increase in the 5-HT concentration in cells of the corticomedullary zone, in the thymocytic parenchyma of the medullary substance of the lobules, in adrenergic nerve terminals, and in the tissue basophils after 15 min. The argentaffin reaction in the thymocytic parenchyma was intensified and the number of alcianophilic mast cells increased. Specific reactions to the antigen, differing in direction, were observed in relation to serotonin in cells of the premedullary zone and in the tissue basophils, in which the heterologous antigen (unlike physiological saline with ink) induced a significant increase in the 5-HT concentration. Similar changes, in different directions, also were found in the macrophagelike cells of the subcapsular zone.

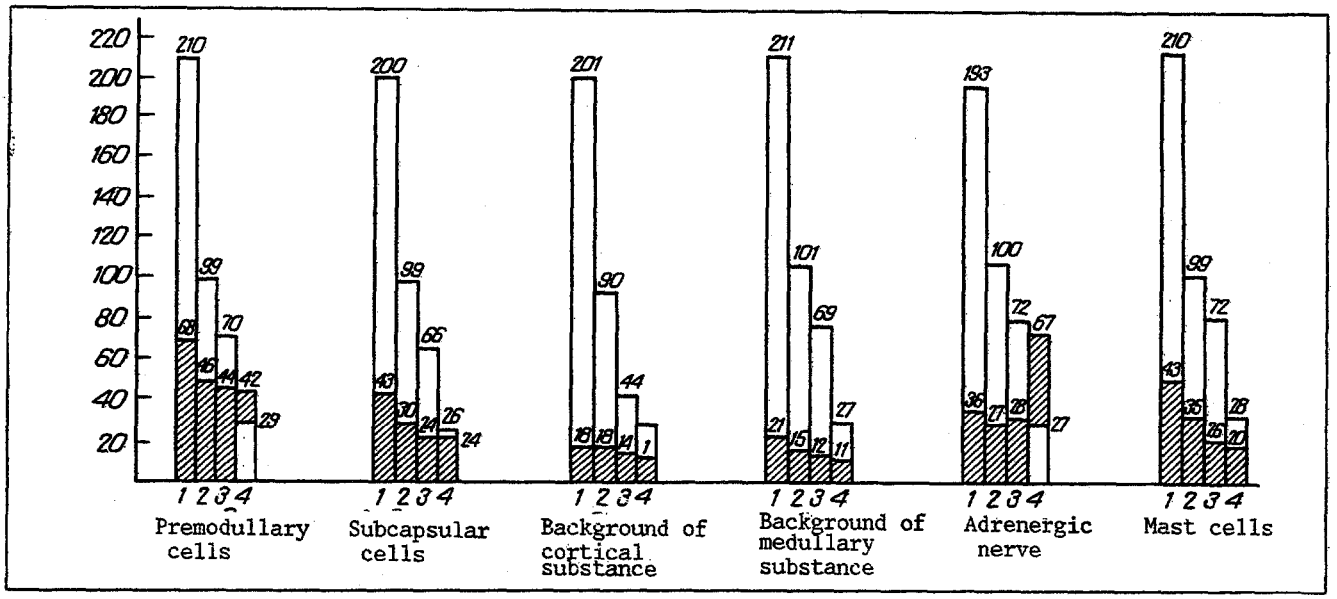

Fig. 2. Distribution of catecholamines (unshaded columns) and serotonin (shaded columns) in structures of intact rat thymus differing in morphology and function. 1) End of May and beginning of June, 2) September, 3) October, 4) November.

An incomplete role of luminescent premedullary cells with diffusely distributed inclusions was found in the lobules 60 min after injection of the corpuscular antigen (in September). Fluorometric analysis of the amine-producing cells of the corticomedullary zone revealed a decrease in the intensity of serotonin luminescence in them (in the intact cells 80 conventional units, 79 in the control, 42 conventional units in the experiments with intravenous injection of SRBC). The decrease in the intensity of luminescence was significant by the Student and Wilcoxon–Mann–Whitney tests. Luminescent mast cells were well demonstrable, but the predominant forms were degranulating: the granules themselves were orange in color and not very bright. The results of fluorometric analysis showed a significant decrease in the 5-HT concentration after immunization with T-dependent antigen.

Quantitative and qualitative measurements revealed considerable seasonal changes in the thymocytic parenchyma of the thymus affecting 5-HT but not CA, and in reactions of both antigenic and nonantigenic type. Whereas in spring (end of May and beginning of June) increased intensity of luminescence of 5-HT was observed 60 min after immunization in the thymocytic parenchyma of the thymus, in September, on the contrary, the numerical values were significantly lower.

The results of experiments conducted in November showed total areactivity of bioamines to the antigenic stimulus not only in the thymocytic parenchyma of the gland, but also in other structures of the thymus.

A significant difference in the intensity of luminescence of CA appeared in the luminescent cells of the subcapsular zone in September, but the same tendency also continued at the end and in the beginning of June. In November the CA level in the subcapsular cells remained unchanged in response to the antigenic stimulus.

In experiments conducted in the fall, the highest level of detection of MAO activity in the cellular structures by Glenner's method was recorded. MAO-positive cells were distributed in a similar pattern to bioamine-containing cells in the corticomedullary zone. The latter formed a complete circle around the medulla in the lobules of the thymus. Around the periphery of the lobules MAO-positive nerve fibers accompanied by mast cells always were found. Injection of the corpuscular antigen led to reduced MAO activity, unlike what was observed in experiments in spring. The same tendency was observed in the thymus with respect to the results of the experiment at the end of October. Direct correlation was found between levels of neurotransmitters and their inactivating enzymes (MAO) in the mast cells. In September, CA and 5-HT were found in high concentrations in these cells by the luminescence-histochemical method, whereas activity of MAO was lowest. In November, the bioamine level in the tissue basophils was lowered, but MAO activity was increased. In response to injection of the corpuscular antigen, many MAO-positive cells were detected, on account of their clearly

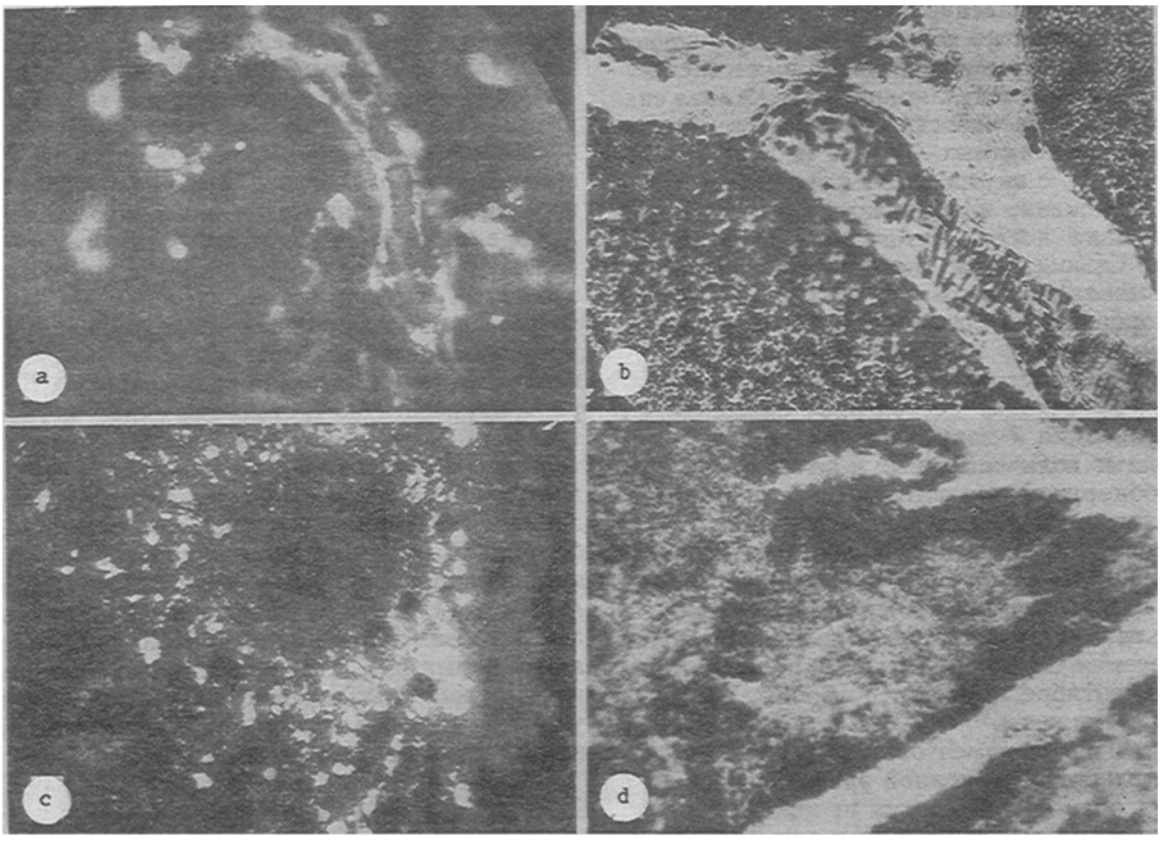

Fig. 3. Adrenergic structures of rat thymus: a) adrenergic nerve fibers along course of blood vessels (Falck's method, UL-2 microscope, objective 90, homal 1.7); b) arterioles in interlobular septa of thymus (Masson's stain); c) luminescent cells in corticomedullary zone (Falck's method, objective 40, homal 1.7, LYUMAM-1A microscope); d) thymic lobule (Masson–Fontana stain, Biolam 70 microscope, objective 10, homal 3).

distinguishable nuclei and their large formazan granules. Compared with the control group of animals, detectability of lipid-containing cells and tissue eosinophils was increased 60 min after injection of SRBC. During antigenic stimulation with SRBC the degree and trend of changes in the status and character of inactivation of neurotransmitters in the thymus were found to have well marked seasonal variations.

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