LITERATURE CITED

1. M. S. Abdullakhodzhaeva and U. A. Aripov, Morphology of Lymphoid Tissue during Allografting and Immunosuppression [in Russian], Tashkent (1980).
2. G. G. Avtandilov, V. P. Nevzorov, and O. F. Nevzorova, Systemic Stereometric Analysis of Cell Ultrastructures [in Russian], Kishinev (1984).
3. N. V. Eletskaya, "Morphology and histochemistry of mouse lymphoid tissue during skin allografting and immunodepressive therapy with batriden," Dissertation for the degree of Candidate of Medical Sciences, Tashkent (1974).
4. Z. Kemileva, The Thymus [Russian translation], Moscow (1984).
5. V. I. Kondror, The Pituitary and Adrenals in Radiation Damage [in Russian], Moscow (1965(.
6. E. A. Korneva, V. M. Klimenko, and Z. K. Shkhinek, Neurohumoral Basis of Immune Homeostasis [in Russian], Leningrad (1978).
7. B. I. Monastyrskaya, The Adenohypophysis [in Russian], Leningrad (1974).
8. G. A. Teshchenko and B. I. Monastyrskaya, Radiobiologiya, 17, No. 1, 45 (1977).
9. Kh. Z. Tursunov, "Morphology of the adrenal cortex and reticuloendothelial cells of the thymus during the development of transplantation immunity," Dissertation for the degree of Candidate of Medical Sciences, Tashkent (1983).
10. V. F. Chebotarev, Endocrine Regulation of Immunogenesis [in Russian], Kiev (1979).

# MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS OF LYMPH NODES, THYROID GLAND, AND TESTES OF RATS DURING STRESS-INDUCED ADAPTATION AND ACTIVATION

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KEY WORDS: adaptive reactions; lymphoid tissue; thyroid epithelium; spermatogenesis

The study of dependence of the character of response of an organism to the factor acting on it has led to the discovery of general nonspecific adaptive responses (GNAR) such as the training response, developing in response to a weak stimulus, and the activation reaction developing in response to a stimulus of average strength [4, 6]. Unlike stress, which is the nonspecific basis of many pathological processes, these GNAR are physiological and are characterized by increased activity of the defensive systems of the body [4, 6]. An important role in this situation is played by inclusion of lymphoid and endocrine organs in the reactive process. The aim of this investigation was to compare quantitatively the morphological and physiological changes taking place in lymph nodes, the thyroid gland, and testes of rats and to establish correlation between characteristic parameters of the state of these organs during the development of a response of stress and activation.

## EXPERIMENTAL METHOD

Responses of stress and activation were induced by subcutaneous injections of adrenalin into 80 noninbred male albino rats weighing 160-170 g, in doses of 125 µg/kg (group 1) and 5 µg/kg (group 2) body weight. The development of the response was signaled by values of blood parameters [5, 7]. The rats were killed by decapitation 2 days after the injections. Lymph nodes, thyroid gland, and testes were fixed in Carnoy's fluid. Paraffin sections 5 µ thick were stained by Brachet's method. Morphometric investigations were carried out with an ocular

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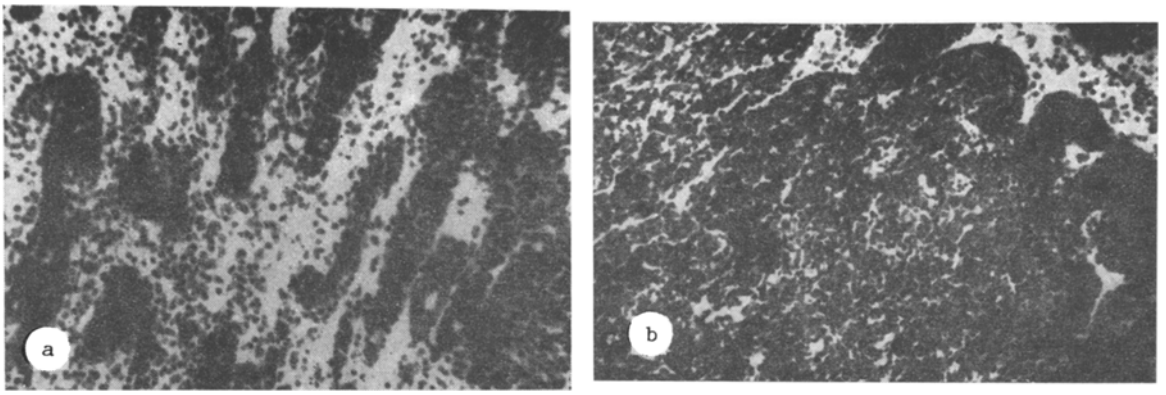


Fig. 1. Lymph node of rat. Branchet's stain, 56 ×. a) Stress. Hypoplasia and degeneration of lymphoid cells in cortex and medulla. b) Activation. Hyperplasia of lymphoid cells of cortex and medulla with differentiation of immature forms into small lymphocytes.

TABLE 1. Morphological and Physiological Parameters of Lymph Nodes, Thyroid Gland, and Testes during Development of Stress and Activation Response (M ± m)

| Organs | Number of variants | Induced responses | |
|---|---|---|---|
| | | stress | activation |
| Lymph nodes | | | |
| Width of medullary cords, μ | 20 | 74±6,5 | 238±18,5* |
| Diameter of follicles, μ | 20 | 191±12,0 | 536±25* |
| RNA concentration in nuclei of small lymphocytes, conventional OD units | 50 | 0,25±0,02 | 0,47±0,01* |
| RNA concentration in nuclei of blast forms, con. OD units | 50 | 0,29±0,01 | 0,17±0,01* |
| Thyroid gland | | | |
| Diameter of follicles, μ | 20 | 112±5,5 | 66±2,0* |
| Height of thyroid epithelium, μ | 20 | 8,1±1,3 | 15,3±2,2* |
| Diameter of nuclei of thyrocytes, μ | 20 | 4,7±0,3 | 7,5±1,1* |
| RNA concentration in thyrocyte nuclei, con. OD units | 50 | 0,90±0,02 | 0,29±0,01* |
| RNA concentration in thyrocyte cytoplasm, con. OD units | 50 | 0,42±0,02 | 0,15±0,01* |
| PBI, μg% | 20 | 2,35±0,93 | 4,57±1,53** |
| BEI, μg% | 20 | 0,54±0,24 | 1,36±0,34** |
| Testes | | | |
| Diameter of tubules, μ | 20 | 171,8±2,3 | 236,1±9,7* |
| Area of tubules, $\mu^2$ | 20 | 24892,9±2041 | 52985,0±4309* |
| Number of spermatogonia per tubule | 20 | 62,40±2,3 | 87,4±3,4* |
| Volume of cells of spermatogonia, $\mu^3$ | 20 | 19,88±3,07 | 44,32±3,0* |
| Number of division figures in primary spermatocytes | 20 | 2,8±0,5 | 4,9±1,9* |
| Number of Sertoli cells per tubule | 20 | 6,3±0,6 | 11,4±0,8* |
| Number of spermatozoa per tubule | 20 | 67,5±2,9 | 240,4±7,1* |

Legend. *$p < 0.001$, **$p < 0.05$.

micrometer. The testes were studied by the method in [9]. The RNA concentration was determined cytophotometrically on an "Amplival" photometer ("Carl Zeiss") at a wavelength of 560 nm. The plasma concentration of protein-bound iodine (PBI) was determined by the method of Barker and Solly, in Stepanov's modification, and that of butanol-extracted iodine (BEI) by the method of Pankov and Usvatova. The results were analyzed by Student's t test and the Wilcoxon–Mann–Whitney U test, and also by determination of coefficients of paired (r) and multiple (R) correlation.

## EXPERIMENTAL RESULTS

Most rats of group 1 developed stress with characteristic blood parameters and hypoplastic changes in the lymph nodes. The follicles were reduced in size because of hypoplasia and destruction of mature lymphoid elements. The medullary cords were reduced in thickness (Fig. 1a). Meanwhile the number of blast forms was increased, probably indicating slowing of the rate of cell maturation. The RNA concentration in the blast cells of the follicles was high, but in the lymphocytes with a narrow rim of cytoplasm, in both follicles and medullary cords, it was low (Table 1). The development of the activation response in the rats of group 2 was characterized by the typical ratio of its white blood cells and by some degree of hypertrophy of the lymph nodes. The dimensions of the corona of the follicles and the

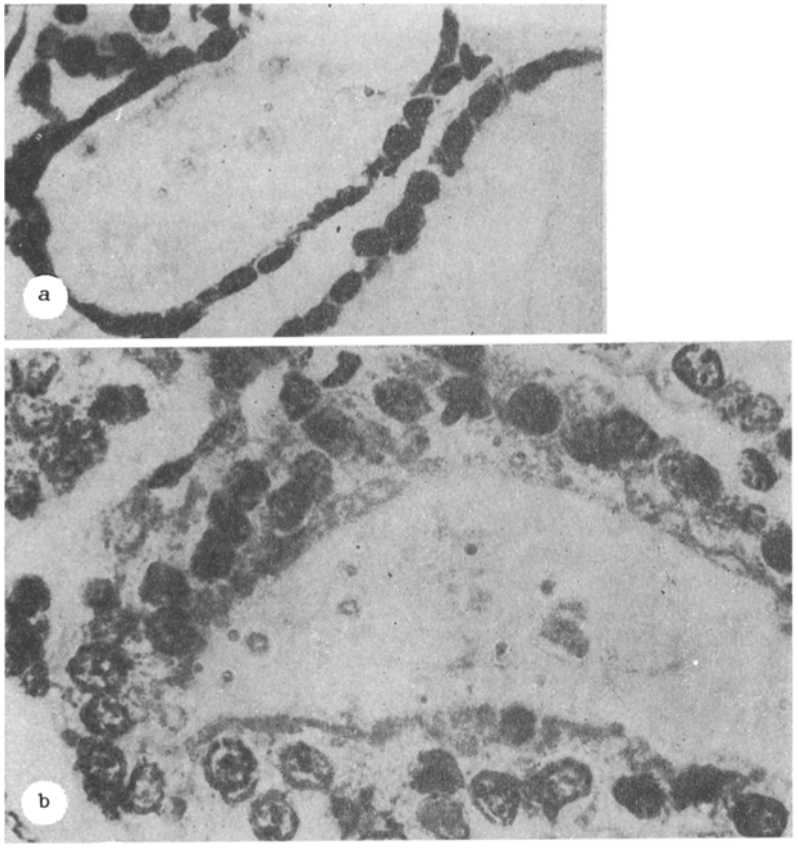


Fig. 2

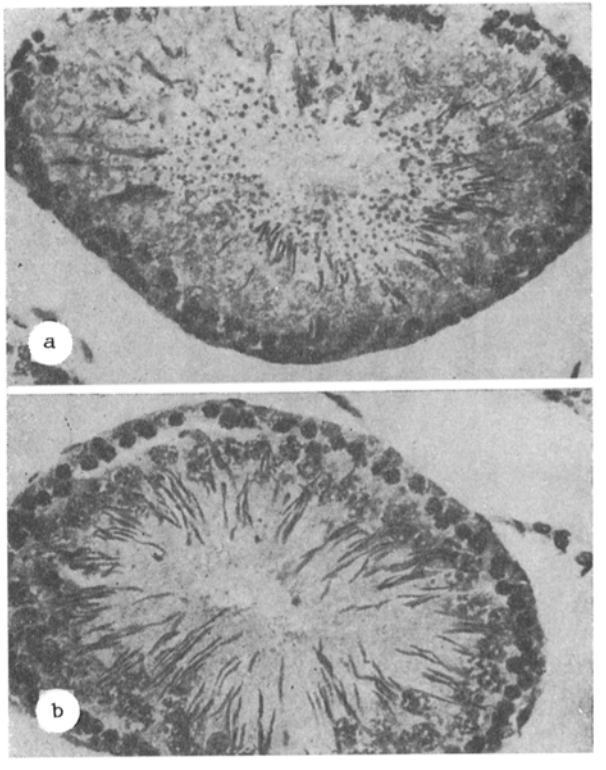


Fig. 3

Fig. 2. Thyroid gland of rat. Einarson's stain. 400 ×. a) Stress. Widening of follicles, increased density of thyroid epithelium; b) activation. Follicles of average and small size, epithelium cylindrical in form, colloid, vacuolated.

Fig. 3. Testis of rat. Einarson's stain. 280 ×. A) Stress. Decrease in number of spermatozoa in tubule; b) activation. Increase in number of spermatozoa in tubule.

width of the medullary cords were considerably increased (Table 1). In some cases the architectonics of the lymph nodes over wide areas was obliterated because of hyperplasia of the lymphoid tissue of the cortex and thickening or even fusion of the medullary cords into conglomerates (Fig. 1b). A significant increase was observed in the RNA content mainly in lymphocytes with a narrow rim of cytoplasm in the follicles and medullary cords, possible evidence of activation of plastic processes [3]. Infiltration of the vascular walls and capsule of the lymph nodes with lymphoid cells was noted.

The microscopic picture of the thyroid gland after exposure to stress revealed evidence of inhibition of its function, in agreement with data in the literature [1, 2, 8]. The follicles were enlarged and most of them were irregular in shape (Fig. 2a). Thickening of the thyroid epithelium and, in some cases, its desquamation were observed. The RNA concentration in the pycnotic nuclei and cytoplasm of the thyrocytes was increased (Table 1). The colloid was thickened, with solitary resorption vacuoles. Plasma PBI and BEI levels were lowered. Development of the activation response was accompanied by marked stimulation of thyroid function, but without signs of hyperthyroidism. The follicles were of average size and the thyroid epithelium was cylindrical in shape (Fig. 2b). The RNA content in the nuclei and cytoplasm of the thyrocytes was slightly reduced. The intensity of staining of the colloid was greatly reduced, and numerous resorption vacuoles appeared. Vacuolation also was well marked in the cytoplasm of the thyrocytes and in cells of the interfollicular tissue, evidence of increased resorption of secretion. A significant increase in concentrations of PBI and BEI in the blood plasma was observed (Table 1).

In the testes of rats exposed to stress, tubules with a small diameter and with very few spermatozoa, Sertoli cells, and spermatogonia predominated (Table 1). In some tubules no spermatozoa whatever could be identified. At all stages of maturation of the sex cells dystrophic changes were found in the cytoplasm and nuclei, with accumulation of detritis (Fig. 3a). The intensity of the response to RNA was low. The presence of these features points to inhibition of spermatogenesis. During the development of the activation response, the majority of the tubules in the testes of the rats increased in size and diameter (Fig. 3b). The number of spermatozoa, Sertoli cells, and spermatogonia also increased. An increase was observed in the number of division figures in the primary spermatocytes. The intensity of the reaction for RNA in the spermatogonia was high.

Statistical evaluation of changes obtained in response to injection of adrenalin in doses of 125 and 5 μg/kg revealed not only a difference in the parameters on the greater–smaller scale, but also in the character of the relationship between them. For instance, when correlation was assessed between parameters of activity of the thyroid gland (PBI, BEI), and lymph nodes (RNA) and the number of lymphocytes in the blood, using an algorithm of correlation analysis, a significant increase in the strength of correlation was observed when a small dose of adrenalin was used ($R = 0.99$). This substantiates the choice of certain prognostic indicators from the whole set available, characterizing activity of the endocrine and thymico-lymphatic systems during goal-directed maintenance of physiological GNAR. The discovery of close correlation between the parameters along the lines of intersystem relations is evidence of the high degree of coordination of the changes in functional activity of the organs chosen for study during the development of the activation response, and this may be responsible for the corrective influence of this response in connection with therapeutic and prophylactic procedures.

## LITERATURE CITED


1. B. V. Aleshin and V. I. Gubskii, The Hypothalamus and Thyroid Gland [in Russian], Moscow (1983).
2. M. G. Amiragova and M. P. Arkhangel'skaya, Byull. Éksp. Biol. Med., No. 6, 24 (1982).
3. Yu. I. Borodin and V. N. Grigor'ev, The Lymph Node in Circadian Disturbances [in Russian], Novosibirsk (1986).
4. L. Kh. Garkavi, "Adaptive response of 'activation' and its role in the mechanism of the antitumor effect of hypothalamic stimulation," Author's abstract of dissertation for the degree of Doctor of Medical Sciences, Donetsk (1968).
5. L. Kh. Garkavi, E. B. Kvakina, and M. A. Ukolova, Regulation of Energy Metabolism and Resistance of the Organism [in Russina], Pushchino (1975), pp. 172-182.
6. L. Kh. Garkavi, E. B. Kvakina, and M. A. Ukolova, Adaptive Reactions and Resistance of the Organism [in Russina], Rostov-on-Don (1977).

7. L. Kh. Garkavi, E. B. Kvakina, A. K. Mulatova, and A. I. Shikhlyarova, Vopr. Onkol., No. 6, 48 (1980).
8. L. Kh. Garkavi, E. B. Kvakina, and E. A. Sheiko, Probl. Éndokrinol., No. 1, 45 (1987).
9. Yu. I. Ukhov and A. F. Astrakhantsev, Arkh. Anat., No. 3, 66 (1983).
10. "The evolution of the stress concept," Sci. Am., 62, No. 6, 642 (1973).

# AGE CHANGES IN FIBRONECTIN LEVELS OF THE DRAINAGE SYSTEM OF THE EYE

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Fibronectin is an extracellular adhesive glycoprotein which can bind selectively with type II collagen, the main constituent of the interstitial tissue of the trabecular system of the human eye [8], it can increase resistance to the outflow of aqueous humor [4, 5], and can thus raise the intraocular pressure. Fibronectin is found in the drainage system of healthy adults [4]. It is not clear, however, whether the content of this protein changes with age. This is an important concept in connection with the sharp increase observed in the fibronectin content during progression of primary open-angle glaucoma [2].

In the investigation described below the content of fibronectin in the trabecular apparatus of the human eye was studied in individuals aged between 49 and 76 years.

## EXPERIMENTAL METHOD

The investigation was conducted on eight eyes from four donors aged from 49 to 76 years, and free from eye diseases. The eyes were enucleated 5-7 h after death and fixed in 4% formaldehyde in phosphate buffer, pH 7.2-7.4. The perilimbic regions of the sclera, containing the drainage zone, the cornea, sclera, and ciliary muscle fibers were isolated. Fibronectin was detected in serial transverse paraffin sections (5-6 μ) of the trabecular zone by the indirect immunoperoxidase method [3]. The sections were incubated consecutively with rabbit antibodies to human fibronectin after which the complex was revealed with a conjugate of anti-immunoglobulin antibodies and peroxidase. Peroxidase activity was found with the aid of a substrate mixture containing $H_2O_2$ and diaminobenzidine. The reaction was recorded on a "Vickers M-86" scanning integrating microdensitometer (20 × objective, No. 3 probe, wavelength 550 nm, scanning time 5 sec). Rabbit antibodies to type I herpes simplex virus served as the control.

## EXPERIMENTAL RESULTS

The study of sections of enucleated normotensive eyes revealed fibronectin in the form of amorphous, diffuse yellowish brown deposits. Staining extended not only along the drainage pathways (trabecular network, canals of Schlemm, collectors, venous vessels of the sclera), but also to surrounding structures. The latter include the sclera, the intermuscular spaces of the ciliary muscle, and the cornea. The intensity of staining was greater, on visual examination, in the endothelial lining of the canal of Schlemm, the collectors, and veins. Staining of the inner wall of the canal of Schlemm was brightest, that of the trabecular ap-

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