MINISTRY OF HIGHER EDUCATION, SCIENCE AND INNOVATIONS OF THE REPUBLIC OF UZBEKISTAN

MINISTRY OF HEALTH CARE OF THE REPUBLIC OF UZBEKISTAN

FERGANA MEDICAL INSTITUTE OF PUBLIC HEALTH

HISTOLOGY AND BIOLOGY DEPARTMENT

M.T. Yuldasheva

Modern Concepts of Structural and Functional Interrelations Between the Thymus and the Thyroid Gland

(Monograph)

Ferghana – 2025

This monograph summarizes facts about the development of pronounced thymic hypoplasia under conditions of thyroid hormone deficiency during the prepubertal period, which may serve as a basis for the development of effective methods of prevention and treatment for children and adolescents with various thyroid dysfunctions. The results of the study can also be used in the educational process at medical institutions in courses on histology, pathological physiology, and endocrinology, when discussing immune-endocrine relationships.

Reviewers:

H.M. Aliyev	PhD, Associate Professor of Medical Biology and Histology department of Andijan State Medical Institute
Fattaxov N. X.	DSc, professor Head of the Department of faculty and hospital surery, DSc

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Introduction

As evolution progresses, new mechanisms of coordination emerge within the organism, fostering structural and functional interconnections. These processes have facilitated the integration of various organ and system functions. One notable example of such integration between the immune and endocrine systems has been explored in numerous studies [Robinson M. V., Obut T. A., Melnikova E. V., Trufakin V. A. Experimental hyperthyroidism: cellular and humoral immunity indicators and their correction // Bulletin of Experimental Biology and Medicine - 2013. - Vol. 156, No. 7. - P. 460-462] [5; p. 45-51, 10; p. 460-462, 19 - p. 410-411].

It is well-established that damage to endocrine organs triggers specific immune system shifts, which in turn lead to disruptions in endocrine function [Wu K., Zhao M., Ma C., et al. Influence of Thyrotropin on T Cell Development in the Thymus in a Subclinical Hypothyroidism Mouse Model // Scand J Immunol. - 2017. - Vol. 85, No. 1. - P. 35-42.] [6; p. 34-38]. Of particular interest is the relationship between the thyroid gland and the immune system. Research has demonstrated that thyroid hormones such as T4, T3, and thyrotropin stimulate immune cells, including macrophages, dendritic cells, and various T- and B-lymphocyte subpopulations [23, p. 12-22].

Various immune disorders in the body of patients emerge, which are largely dependent on the levels of thyroid and thyrotropin hormones. However, morphological changes in hypo- and hyperthyroid conditions with immune disturbances in different age periods remain poorly studied. Previous research showed that hypo- and hyperthyroid conditions that arose in the prepubertal period lead to negative consequences for the development of endocrine functions in rats. Thyroid diseases are often observed in our region, leading to immune shifts in the body. At present, large-scale health system reforms are widely conducted in our country, which necessitate the development of extensive program activities aimed at early diagnosis of diseases and reducing their negative consequences among various segments of the population. Adolescents are the most active and promising part of any society. At the same time, this is the age when the final formation of all systems of the body and sexual maturation occur, which is clearly considered a physiological stress test for the body. As a result, many diseases that develop at this age are often underestimated both by adolescents themselves and by doctors. The development of the organism is characterized by quantitative and qualitative changes in the parameters of organs and tissues aimed at maintaining homeostasis of the body. Human health is influenced by exogenous and endogenous factors, to which the young organism is especially sensitive [12; p. 363]. The structural and functional alterations in immune system organs caused by thyroid dysfunctions during the prepubertal and pubertal stages of life remain inadequately explored. Therefore, we have chosen to investigate the structural mechanisms underlying immune system disturbances, with a particular focus on the thymus.

In solving these problems, the strategy for the development of New Uzbekistan for 2022-2026 "Improving the quality of life of various segments of the population and medical care through early diagnosis of diseases and the use of modern technologies for their effective treatment" [20; p. 750-755] is of great importance.

This research in some way serves the solution to the tasks reflected in the Laws of the Republic of Uzbekistan "On the Protection of Citizens' Health," "On the Quality and Safety of Food Products," and the Decrees of the President of the Republic of Uzbekistan: PP-2133 of February 19, 2014 "The Year of a Healthy Child," PP-2221 of August 1, 2014 "On the State Program for the Protection of Maternal, Child, and Adolescent Health in Uzbekistan for the Period 2014-2018," PP-4947 of February 7, 2017 "Strategy for Actions on Five Priority Areas of Development of the Republic of Uzbekistan in 2017-2021," the strategy for the development of New Uzbekistan for 2022-2026 of January 28, 2021, No. UP-60, and other regulatory legal documents adopted in this field [Strategy for the development of New Uzbekistan for 2022-2026 of January 28, 2021, No. UP-60][30; p. 1051-1061].

Research by scientists from neighboring countries [6; p. 34-38, 10; p. 460-462, 11; p. 734-736, 24; p. 25-29, 27; p. 62-67] has shown a close connection between thyroid hormones and the immune system. Studies have shown that patients and experimental animals with various thyroid dysfunctions exhibit alterations in cellular and humoral immunity markers. However, the results are often conflicting and sometimes contradictory. Many researchers have noted a connection between blood thyroid hormone levels and various changes in the immune system. In instances of autoimmune thyroiditis (Hashimoto's disease), where hypothyroidism or hypothyroxinemia occurs, there is a decrease in the overall lymphocyte count, disruption in the T-suppressor/helper cell balance, abnormal antibody production, and elevated levels of autoantibodies. Experimental models using metamizole, propylthiouracil, studied the effect of hypothyroidism on the immune system. Only a few experimental studies have revealed dysfunction of the thymus after thyroidectomy in rats [4; p. 154-157, 5; p. 45-51, 6; p. 34-38]. Sometimes immune shifts are detected in patients with hyperthyroidism, which is more commonly observed in Graves' disease. However, this type of immune shift is less pronounced and, in many cases, is reflected in an imbalance between the T- and B-immune systems.

At the same time, significant thymic enlargement and its hyperplasia are often observed. Experimental studies on a potassium iodide model showed that hyperthyroidism causes an increase in the weight and cellularity of the thymus and spleen, and a change in the ratio of T-lymphocyte subpopulations [10; p. 460-462].

Immuno-endocrine relationships in physiological and pathological conditions in the Republic of Uzbekistan have been comparatively little studied. In the works of Tukhtaeva K. R., Abdurakhmanova M. A. (2001, 2002), a correlation between structural changes in the thymus and adrenal glands under conditions of experimental toxic liver damage by heliotrin was demonstrated [1; p. 34]. Studies by domestic researchers who experimentally reproduced hypo- and hyperthyroid conditions in prepubertal and sexually mature rats observed spermatozoa functions [19; p. 22].

Thus, despite the existence of convincing evidence of the significant role of thyroid hormones in regulating immune genesis, the structural-functional mechanisms of immune disorders in various thyroid dysfunctions remain unexplored. In this regard, our laboratory has been conducting studies for several years on the state of the thymus under hypo- and hyperthyroid conditions reproduced at various periods of postnatal ontogenesis [2; p. 28-31, 3; p. 7-8, 4; p. 12-13, 17; p. 159-160, 18; p. 11].

Chapter I. Structural-functional features of the thymus in thyroid dysfunctions

§1.1. Modern views on the structure and function of the thymus in mammals

Research results on the thymus of various vertebrates, including mammals, using modern ultrasonic, morphological, radiographic, immunoenzymatic, and biochemical methods have provided new data on the structural-functional organization of the organ. These results are supported by scientific works of academician K. A. Zufarov, as well as his students (K. R. Tukhtaev, 1987; U. D. Jalalov, 1990; M. Kh. Rakhmatova, 1998; F. Kh. Azizova, 2001; M. Abdurakhmanov, 2001; P. R.), who studied this mysterious organ in ontogenesis and phylogenesis. The use of a wide range of research methods: physiological, biochemical, immunohistochemical, and morphological indicators, has led to obtaining new results about the structural-functional variability of the thymus [1; p. 34, 2; p. 36, 9; p. 20].

According to most scientists, the thymus (Tm) is the least studied organ, which undergoes changes in size and morphological transformations throughout a person's life. In childhood, the thymus and its immuno-neuroendocrine interactions with other organs play a leading role, as they ensure normal development, supporting immunological protection against various environmental factors [8; p. 232, 9; p. 20, 14; p. 784-788]. In thymus pathology, a high level of complications in children is observed, associated with immune dysfunction.

It is well known that the thymus is a lymphoepithelial organ, the parenchyma of which is epithelial tissue. The thymus itself has a triangular shape and usually consists of two lobes. Each lobe is divided into numerous incomplete lobules by layers of connective tissue. In these lobules, the cortical and medullary zones can be distinguished, differing in cellular composition and functions. According to many researchers, in the cortical zone, one can find mainly immature lymphocytes (thymocytes), and in the medullary zone, differentiated T-lymphocytes that have undergone the maturation process. For full maturation, T-lymphocytes need interaction with various cellular and humoral factors, including thymic hormones, as well as factors of the neuroendocrine system, which includes thyroid hormones.

It was established that the pituitary gland plays a significant role in the formation and growth of the thymus, as evidenced by thymic involution resulting from hypophysectomy. It is also known that somatotropin accelerates the incorporation of 3H-thymidine into the nuclei of proliferating populations of thymocytes. Furthermore, the introduction of somatotropin contributes to the restoration of the normal structure of the thymus in hypophysectomized animals. At the same time, after the administration of hydrocortisone, the recovery of thymus mass and the number of thymocytes occurs at the expense of the proliferation of cortisone-resistant thymocytes that do not have receptors for somatotropin [21; p. 410-411].

In pharmacological hypoplasia caused by the administration of thymalin and the onset of thymic insufficiency, there is an activation of the corticotropic function, accompanied by the suppression of the somatotropic and prolactin-secreting functions of the adenohypophysis. This is accompanied by the stimulation of steroid hormone production in the adrenal cortex [13; p. 316]. In the case of thymus hypertrophy [33; p. 1051-1061], an opposite picture of the relationship between the pituitary and adrenal cortex is observed. The introduction of complex thymic peptides into intact animals contributes to an increase in the secretion of ACTH, STG, prolactin, and corticosteroids. The ambiguity of the relationship between the thymus and the adrenal cortex is manifested in that the increase in corticosteroid levels is observed after the removal of the thymus. In the absence of the thymus, blood plasma levels of thyroid hormones—thyroxine and triiodothyronine—decrease, whereas transplanting the thymus into older animals results in an increase in thyroxine levels in the plasma [18; p. 11-40]. Administering thymus extracts helps prevent the onset of endocrine disorders, not only when given immediately after thymectomy but also during the later stages, after immunodeficiency has already developed. The functional status of the thyroid gland, adrenal cortex, and testes endocrine apparatus was assessed during thymalin treatment in animals with both intact immune systems and those with secondary immunodeficiency induced by thymectomy. The results indicated that two months after thymectomy, signs of diminished thyroid function, along with hyperplasia of steroid-producing cells in the testes and adrenal cortex, were evident [8; p. 232, 9; p. 20].

The thyroid gland plays a crytical role in the organism's development and the regulation of numerous metabolic processes throughout life. Thyroid hormones and their inhibitors are widely utilized to adjust processes associated with the organ's dysfunction. Growing evidence suggests that thyroid hormones —thyroxine (T4) and 3,3,5-triiodo-L-thyronine (T3)— and their inhibitors are modulators of the immune response. [16; p. 59-61]

M.V. Robinson and co-authors studied the lymphoid tissue of intact mice during hyperfunction (administration of thyroxine) and hypofunction (administration of thyroxine inhibitor mercazolil) of the thyroid gland. The study results showed that the administration of mercazolil led to an increase in spleen mass and cellular composition of the thymus and spleen. Thyroxine had no effect on these parameters. The number of antibody-producing cells in the spleen increased under the influence of mercazolil and thyroxine. Mercazolil increased the number of CD3+, CD4+, CD8+ cells, while thyroxine increased only the CD4+ cells in the thymus. Thus, the administration of mercazolil and thyroxine caused pronounced changes in the cellular and humoral immunity indicators. A significant regulatory effect of the thyroid gland on T-cells and their mediators, on the processes of T-cell migration, and on T- and B-cell differentiation was also revealed [11; p. 460-462, 12; p. 734-736].

Focusing on the study of cellular and humoral immunity in animals with experimental hyperthyroidism, M.V. Robinson caused an increase in the mass and cellularity of the thymus and spleen, increasing the number of antibody-producing cells against EB, and changed the percentage of T-lymphocyte subpopulations by administering potassium iodide solution [11; p. 460-462, 12; p. 734-736].

Thyroid hormones (TH) during pregnancy make a significant contribution to cellular differentiation and development in several tissues of the offspring, primarily the central nervous system (CNS). Deficiencies of TH, such as hypothyroidism or hypothyroxinemia, are very common during pregnancy worldwide and are known to be harmful to fetal development. On the other hand, little is known about the consequences of thyroid hormone (TH) deficiency in the immune system of offspring, with the prevailing belief that the effects are reversible and will only temporarily affect the number of B- and T-cells [21; p. 410-411].

To determine the effect of thyroid hormones on the immune status of the body, experiments were conducted on dogs. Hypothyroidism is one of the most common endocrine diseases in dogs and is usually considered autoimmune in nature. In human hypothyroidism, the thyroid gland is destroyed by both cellular (i.e., autoreactive helper and cytotoxic T-lymphocytes) and humoral (i.e., autoantibodies specific to thyroglobulin, thyroxine, and triiodothyronine) effector mechanisms. The aim of this study was to assess immunological changes in canine hypothyroidism. The study included 28 clinically healthy dogs, 25 hypothyroid dogs without thyroglobulin antibodies, and eight hypothyroid dogs with these autoantibodies. Changes in serum proteins were found in hypothyroid dogs compared to healthy control animals (i.e., elevated levels of α-globulins, β2- and γ-globulins), as well as a higher concentration of acute-phase proteins and circulating immune complexes. Hypothyroid animals had a lower CD4:CD8 ratio in peripheral blood compared to control dogs, and sick dogs also had higher expression of interferon γ (gene and protein expression) and CD28 (gene expression). Similar results were found in both groups of hypothyroid dogs. Thus, canine hypothyroidism is characterized by systemic inflammation with a predominance of cellular immune response.

The immune status, components of the oxidative-reductive system of glutathione, antioxidant enzyme activity, and purine nucleotide metabolism were investigated in animals with experimental hypothyroidism. On the 8th day after an increase in leukocytes in the blood of thyroidectomized rats, lymphocytes, T-helper cells, T-suppressors, and an increased number of B-lymphocytes were found. This was accompanied by a decrease in the activity of adenosine deaminase (AD), AMP deaminase (AMPD), and 5'-nucleotidase (5'N) in the blood, but the AD/AMPD enzyme activity ratio increased. These changes in enzyme activity involved in purine catabolism can be considered as an increase in functional relationships between T- and B-lymphocytes during hypothyroidism [20; p. 138-139, 21; p. 410-411, 24; p. 245-246].

Thyroid hormones thyroxine and triiodothyronine, when administered endogenously, significantly enhance the functional activity of the immune system and various populations of immunocompetent cells, exerting their effect through cytoplasmic and nuclear receptors. This results in a stimulatory impact on the humoral immune response [35; p. 35-42].

The mechanisms underlying the stimulatory effect of thyroid hormones on the humoral immune response remain not fully understood. Some researchers suggest that this influence is primarily realized at the antigen-independent post-thymic stage of T-cell differentiation. The effect on intercellular cooperation in irradiated recipients is only evident when hormones are applied to donors, but not to T-lymphocyte recipients. A significant role of thyroid hormones in supporessing T-suppressor functions has also been identified in these studies. Other researchers propose that thyroid hormones directly stimulate B-cell differentiation, as they do not impact the proliferation or differentiation of peripheral human lymphocytes activated by T-mitogens in vitro, but they do enhance the number of antibody-producing cells in cultures stimulated by B-cell mitogens.

One possible mechanism of thyroid hormones' stimulatory effect on immunocompetent cells is their impact on the quantity and function of epithelial-reticular cells in the thymus. Experiments have shown that repeated administration of triiodothyronine to newborn rats significantly increases the number of epithelial cells in the thymus of 1-month-old rats.

Significant immune dysfunctions are observed in cases of thyroid hormone deficiency. Thyroidectomy has been shown to decrease the humoral immune response [28; p. 12-22, 34; p. 253-257]. In newborn animals, thyroidectomy leads to slowed growth of all lymphoid organs and impaired immune function [5; p. 154-157, 6; p. 45-51].

Immune dysfunctions in thyroid disorders have attracted growing attention from clinicians, endocrinologists, and immunologists due to their significant impact. Endocrine glands play a vital role in immune tolerance, homeostasis, and regulating immune responses throughout life, from the development of immune competence in early childhood to adulthood.

Distribution of animals during the reproduction of experimental hypo- and hyperthyroid conditions remains a key aspect of this research.

Animal Groups	Number of Animals	Abs
I. Control for prepubertal period	17	10
II. Control for sexually mature animals	15	10
III. Short-term hypothyroidism in the prepubertal period	17	6
IV. Long-term hypothyroidism in the prepubertal period	15	8
V. Short-term hyperthyroidism in the prepubertal period	16	10
VI. Long-term hyperthyroidism in the prepubertal period	16	10
VII. Hypothyroidism in sexually mature animals	17	10
VIII. Hyperthyroidism in sexually mature animals	15	10
Total	128	74

The first and second groups (32 rats) were composed of control animals. In particular, Group I (body weight 70-80 g, n=17) served as the control for prepubertal animals, while Group II (body weight over 150 g, n=15) comprised sexually mature rats that acted as the control for the experimental sexually mature animals.

Short-term and long-term hypothyroidism in prepubertal rats was induced by administering Mercazolil. Rats in Groups III–IV were given Mercazolil orally at a dose of 0.5 mg per 100 g body weight for 14 days, followed by a maintenance dose of 0.25 mg per 100 g body weight for one month (short-term hypothyroidism). Subsequently, Group III stopped receiving Mercazolil, while Group IV continued to receive the drug until sexual maturity to reproduce long-term hypothyroidism. The short-term and long-term hyperthyroidism models in the prepubertal period were reproduced in animals of Groups V (n=16) and VI (n=16). At the beginning, these animals were given thyreoidin at a dose of 10 mg per 100 g body weight for two weeks, followed by a maintenance dose of 5 mg per 100 g body weight for one month.

As with the hypothyroidism model, after one month, the administration of exogenous L-thyroxine was stopped in Group V animals, while Group VI continued to receive L-thyroxine until sexual maturity.

We also obtained experimental models in sexually mature male rats (Groups VII-VIII) using Mercazolil and L-thyroxine. To obtain an experimental hypothyroidism model, Mercazolil was used, and hyperthyroid conditions were reproduced by administering exogenous L-thyroxine.

Rats in Group VII were administered Mercazolil per os at a dose of 0.5 mg per 100 g body weight for 45 days, while rats in Group VIII were administered L-thyroxine at a dose of 5 mg per 100 g body weight during the same period. As a result, we obtained both short-term and long-term experimental thyroid dysfunction in the prepubertal period, as well as long-term changes in thyroid hormone imbalance in adult animals.

Today, there are many experimental models that cause thyroid hormonal disturbances (partial or total thyroidectomy, etc.), however, pharmacological influence on the thyroid gland remains the primary method (E. S. Detyuk et al., 1973; M. P. Pavlovsky et al., 1986). Nevertheless, the pharmacological interventions we conducted required proof of the presence of specific hormonal shifts in the bodies of the studied animal groups.

In the rats' serum, the levels of tri- and tetraiodothyronine (T3 and T4), as well as thyrotropin (TSH) hormones, were determined using an immunoassay method. These studies were conducted in the laboratory of the "Doctor D" clinic, and we express our sincere gratitude to the clinic's director, Ph.D. D. S. Irgashev.

§2.2. Research Methods

After euthanizing the animals, the thymus was weighed, and its total mass (in mg) and the ratio to body weight (mass index) were determined. After determining the mass of the thymus (for light-optical studies), it was then fixed in Carnoy's or Bouin's fluids, then embedded in paraffin. Hematoxylin and eosin staining was used for sectioning.

On the preparations obtained by the morphometric method of Avtandilov G. A., using a morphometric grid, the absolute and relative areas of different structural-functional zones of the thymic lobes and its connective tissue components were determined.

The cell elements of various structural-functional zones of the cortical and medullary areas were counted both on semi-thin (1 μm) epon-araldite sections stained with basic fuchsin and methylene blue, as well as on thin sections stained with eosin and hematoxylin. Destructive and mitotically dividing thymocytes in different zones of the thymus were also counted on these sections.

For electron microscopy, thymus preparations from various areas were fixed in a 1.25% glutaraldehyde solution (in phosphate buffer, pH 7.3 for one hour) followed by postfixation in a 1% osmium tetroxide solution (in the same buffer for two hours). After washing in buffer solutions and dehydration in increasing concentrations of alcohols and absolute acetone, the material was embedded in a mixture of epon and araldite. Ultra-thin sections were made using an ultramicrotome, and were sequentially contrasted with lead citrate and uranyl acetate, then viewed in an electron microscope.

All data were processed using variance statistics with the computer program package. Differences were considered significant if $P<0.05$.

Chapter III. Thyroid hormone levels, morphometric, and ultrastructural features of the thymus in experimental hypothyroidism induced in the prepubertal period

§3.1. Control animals

Before discussing the structural changes of the thymus in cases of thyroid dysfunction, it is important to first outline the key morphological characteristics of the thymus in control rats of various ages. The morphology and ultrastructure of the thymus in sexually mature rats have been extensively documented in previous studies by our colleagues (M. Kh. Rakhmatova, 1998; M. Abdurakhmanov, 2001; F. Kh. Azizova, 2001). Therefore, our primary focus is on the thymus of prepubertal rats in the control group.

It is worth noting that in rats of this age, the thymus is a fully developed organ with a distinct lobular structure. Externally, the thymus is enclosed in a connective tissue capsule, and its parenchyma is divided into 7–10 lobes by connective tissue septa. These lobes are differentiated into two main regions: the peripheral dark (cortical) zone and the central light (medullary) zone. The distinction between these regions is based on the density of thymocytes (Tc) present in these areas of the lobes, as illustrated in Figure 3.1.

The cortical zone occupies the majority of the lobes and is characterized by a dense distribution of thymocytes. In the subcapsular zone, the lobes primarily consist of less differentiated lymphocyte forms—relatively large cells, identified as (Lbl) and (Plc). As we move deeper into the lobes, especially at the transition between the cortical and medullary zones, the predominant cells become more mature thymocytes, which are characterized by smaller and medium-sized cells.

This detailed description of the thymus in prepubertal rats serves as a baseline for understanding how thyroid dysfunction might influence the structure and function of this vital organ. Since the thymus plays a crucial role in immune system development and function, any morphological changes, such as those potentially induced by thyroid hormone imbalances, could have significant implications for immune responses in these animals.

In summary, the thymus in prepubertal rats is a fully formed organ with a well-defined lobular architecture, exhibiting distinct cortical and medullary zones. These zones are distinguished by the density and maturation of thymocytes, which serve as important markers of the organ's function and structure. By understanding the normal morphology of the thymus in control rats, we can better assess the potential effects of thyroid dysfunction on this organ's structure and function in future studies.

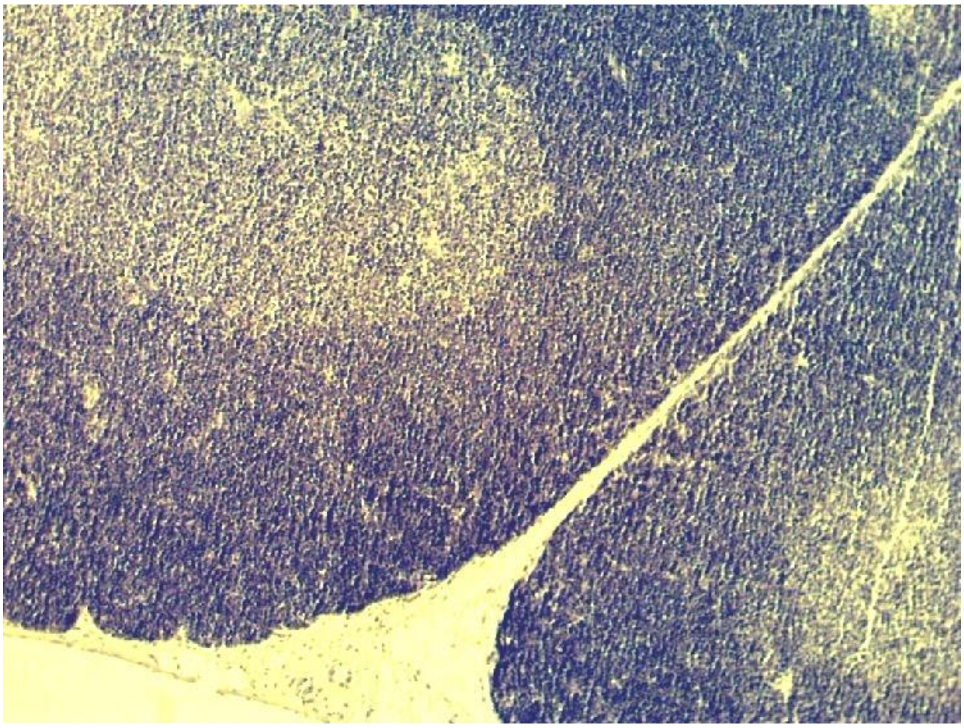


Fig. 3.1. Thymus of a control prepubertal rat.
Capsule, interlobular septum, cortical and medullary zones. Staining with hematoxylin and eosin (H&E). Magnification: 20x, scale bar: 10 µm.

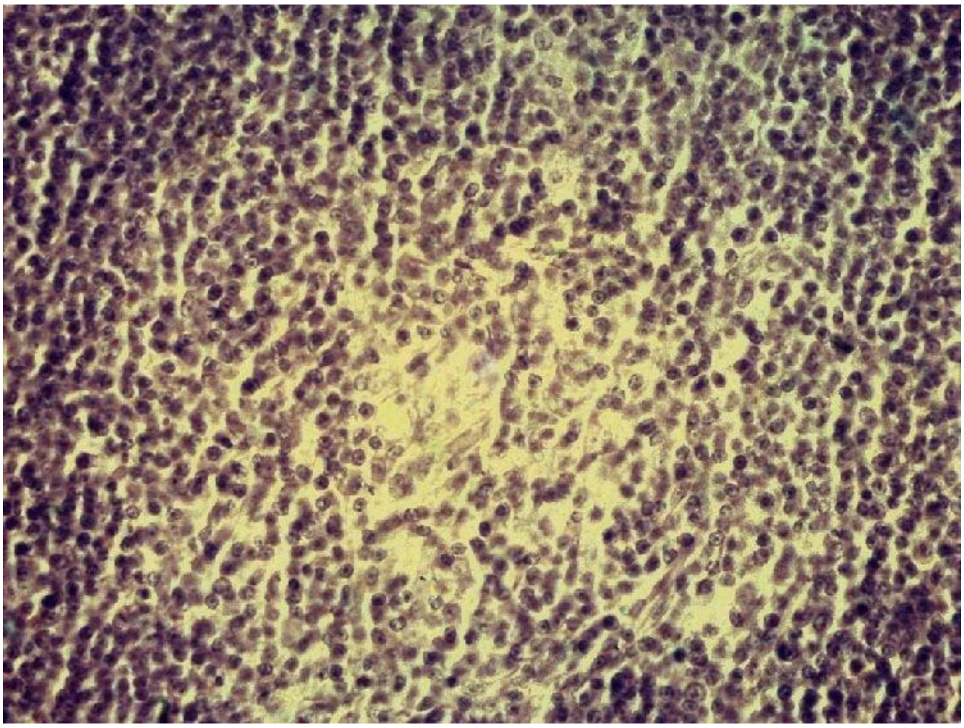


Fig. 3.2. Thymus of a control prepubertal rat.
Medullary zone with Hassall's corpuscles. H&E staining. Magnification: 20x, scale bar: 10 µm.

Lymphocytes (Fig. 3.2.). The cells of the thymic microenvironment are represented by epithelial-reticular cells (ERC), between the processes of which small and medium lymphocytes are located, along with occasional macrophages. Macrophages are found in small quantities around blood capillaries that have flattened endothelium. These immune cells play a crucial role in detecting and responding to various pathogens and foreign particles within the body. In the thymus, the boundary between the medullary and cortical zones of the thymic lobes is referred to as the corticomedullary zone. This region contains a combination of small blood capillaries and postcapillary venules with high endothelium, which are specialized vessels that facilitate the migration of immune cells between the bloodstream and the thymus.

Thymocytes, or developing T-cells, in this zone are primarily composed of medium-sized lymphocytes and smaller, mature lymphocytes, as illustrated in Figure 3.3. Additionally, within the corticomedullary zone, individual macrophages can be observed surrounding the blood capillaries. These macrophages are noticeably larger compared to the ones found in the cortical zone, likely due to their increased activity and the need to perform more extensive immune surveillance and antigen presentation in this region. Additionally, tissue basophils or mast cells (MC) were also observed in this area, contributing to the immune response by releasing histamine and other mediators that influence inflammation and immune cell function.

The medullary zone of the thymus, although occupying a small portion of the entire thymic lobe, is a crucial area for the development and maturation of thymocytes. The characteristic feature of the medullary zone is the relatively low density of lymphocytes present in comparison to the cortical zone. Figure 3.2 visually represents this distinction, depicting the lower density of lymphocytes within the medullary zone. The lymphoid cells present in this area are primarily composed of mature lymphocytes, which include a mix of large, medium, and small forms. This maturation process is essential for producing fully functional T-cells that will later contribute to the immune response.

Due to the low concentration of lymphocytes, the medullary zone allows for the clearer identification of epithelial-reticular cells (ERCs), which have relatively light cytoplasm and large sizes compared to lymphocytes. These ERCs play a vital role in supporting thymocyte development by providing a structural framework and releasing signaling molecules that direct thymocyte maturation. Within this zone, around the blood vessels, individual plasma cells (Pl) and macrophages can also be observed. These cells contribute to immune function in the thymus by clearing debris and pathogens and producing antibodies when necessary.

A distinguishing feature of the medullary zone is the presence of structures known as Hassall's corpuscles. These are formed by concentric layers of keratinized epithelial cells and are visible in certain thymic lobes, as illustrated in Figure 3.2. Hassall's corpuscles are believed to regulate T-cell selection and promote immune tolerance. Under certain conditions, particularly when the thymus is damaged or disrupted, these corpuscles may exhibit degenerative changes, with individual cells within them showing signs of destruction. Such alterations in Hassall's corpuscles can serve as indicators of an ongoing immune response or an adaptive process within the thymus.

Although there are differences in organ weight and thymus mass index between prepubertal and sexually mature male rats, the overall morphology of the thymus remains largely unchanged between these two groups. The thymus in prepubertal male rats maintains a structure similar to that of sexually mature rats, suggesting that despite variations in size and weight across developmental stages, the fundamental architecture and cellular organization of the organ remain consistent. This indicates that the structural integrity and functional capacity of the thymus are already well established before puberty, ensuring that T-cell development and maturation proceed efficiently during early life.

In conclusion, the structural characteristics of the thymus in prepubertal rats—including the organization of the corticomedullary zone, the distribution of thymocytes, macrophages, and other immune cells, as well as the presence of Hassall's corpuscles—serve as a crucial foundation for understanding thymic function in immune development. These findings provide a framework for future research on the impact of thyroid dysfunction on thymic morphology and immune system development, particularly in relation to immune disorders and autoimmune diseases.

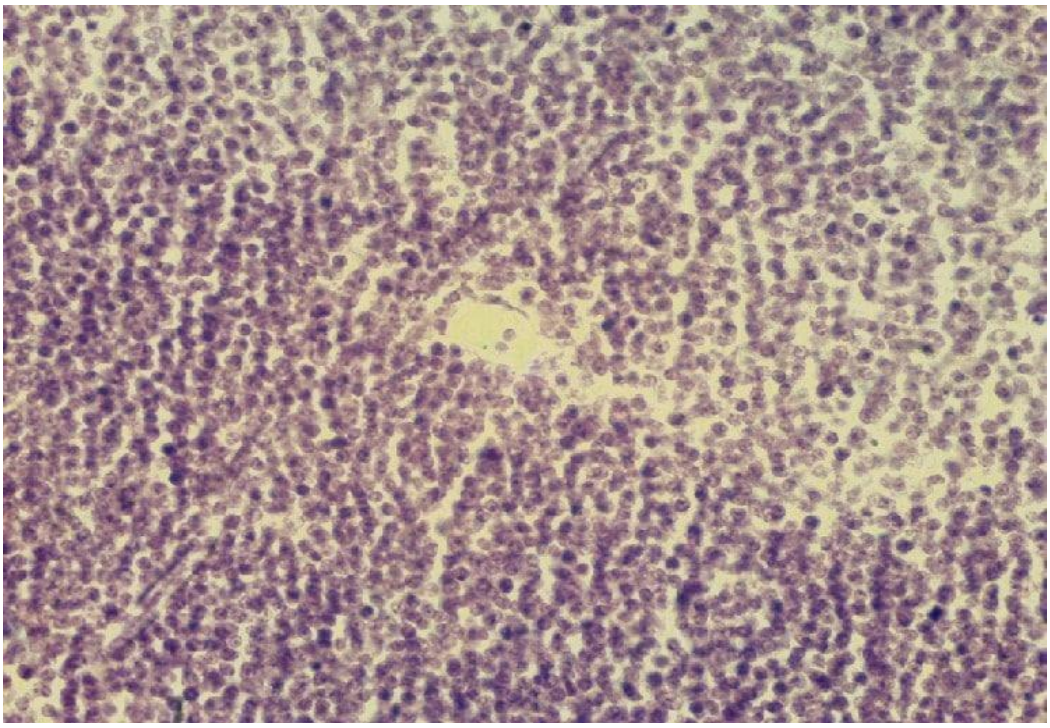


Fig. 3.3. Thymus of a control prepubertal rat.
Corticomedullary zone with capillaries and venules. H&E staining.
Magnification: 20x, scale bar: 10 μm.

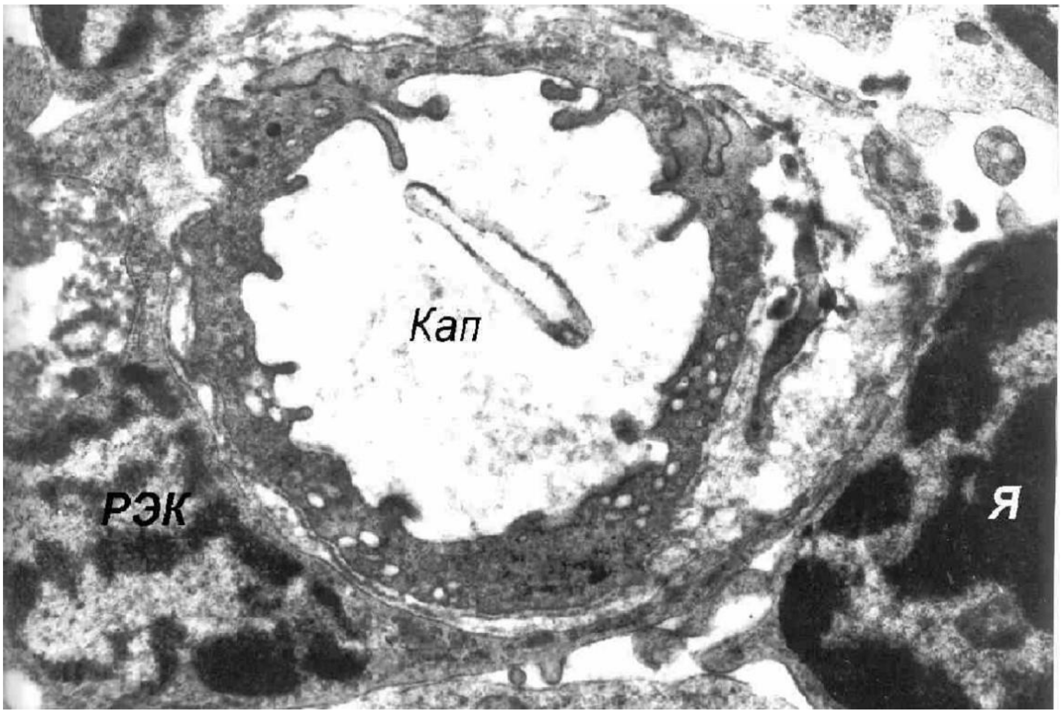


Fig. 3.4. Thymus of a control prepubertal rat.
Blood capillary in the corticomedullary zone. Transmission electron microscopy (TEM). Magnification: x12,500.

Electron microscopy of the thymus in prepubertal rats revealed no significant structural differences when compared to the thymus of sexually mature animals. Blood capillaries were commonly observed in both the cortical and corticomedullary zones (Fig. 3.4.). The endothelial cells of these capillaries exhibited numerous pinocytotic vesicles, suggesting active transendothelial transport. Surrounding the capillaries, structures of the blood-thymus barrier were typically present, consisting of a basal membrane, intercellular material, and processes from epithelial-reticular cells (ERCs) (Fig. 3.4.).

In the cortical zone, ERCs exhibited irregular shapes due to the presence of numerous processes that extended deeply between thymocytes, which predominantly displayed the ultrastructure of large, medium, and small lymphocytes. A key feature of these ERCs was the presence of secretory vacuoles and tonofibrils within their cytoplasm (Fig. 3.5.). The vacuoles varied in size, with some containing fine, dispersed material. ERCs in the medullary zone were distinguishable by larger secretory vacuoles and thicker bundles of tonofibrils compared to those in the cortical zone.

Monocytes and their differentiated forms, macrophages, were among the most frequently identified cell populations within the thymic microenvironment. The highest concentration of macrophages was observed in the cortical zone, whereas their presence in the corticomedullary and medullary zones was comparatively lower. These thymic macrophages exhibited structural features characteristic of their cell type. The nuclei of the macrophages were irregularly shaped due to indentations and protrusions, often containing a visible nucleolus (Fig. 3.6.). The cytoplasm of these macrophages contained numerous primary and secondary lysosomes, which varied in shape and size. Occasionally, large heterophagosomes containing remnants of destroyed thymocytes were observed within the cytoplasm of some cortical and medullary macrophages.

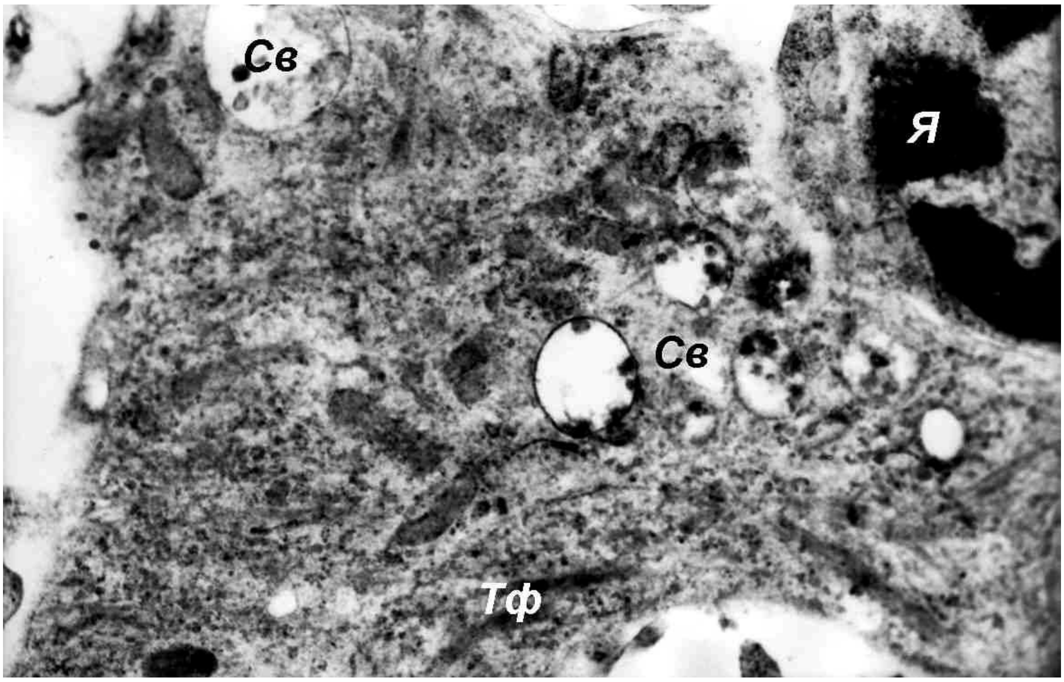


Fig. 3.5. Thymus of a prepubertal control rat.
Epithelioreticular cell (ERC) within the cortical zone.
Transmission electron microscopy (TEM), magnification x24,000.

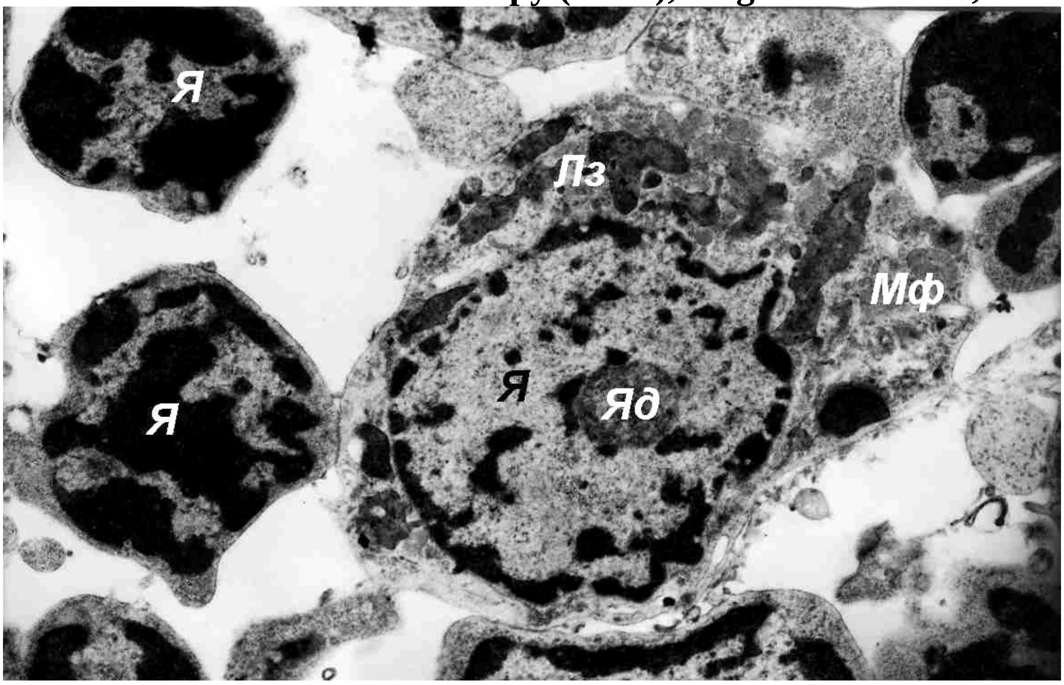


Fig. 3.6. Thymus of a prepubertal control rat.
Macrophage in the cortical zone. TEM, x12,500.

The thymus of prepubertal control animals demonstrates a well-defined lobular organization. Each thymic lobe consists of three distinct regions: the cortical zone (CZ), the corticomedullary zone, and the medullary zone (MZ). These regions are classified based on the density of developing thymocytes and the presence of specialized microenvironmental cells that support their maturation. Although the corticomedullary zone serves as a transitional area, it is less clearly defined under light microscopy.

In comparison, the cortical and medullary zones are more easily distinguishable, serving as key structural features for thymic analysis. This clear distinction facilitates accurate morphometric assessments, including measurements of thymocyte density and identification of various cellular components within the thymic microenvironment. These structural differences are crucial for understanding the maturation process of thymocytes and the functional role of the thymic stroma.

Interestingly, the thymic structure in prepubertal male rats is largely comparable to that in sexually mature individuals, suggesting that the essential framework for thymocyte development and immune function is already in place before puberty. The cortical zone (CZ) is densely packed with thymocytes at different stages of differentiation, where crucial processes such as positive selection take place—ensuring that developing T-cells can recognize self-antigens. The CZ has a higher cellular density compared to the medullary zone (MZ), aiding in its identification. Additionally, epithelial-reticular cells (ERCs) within this region provide structural support and secrete signaling molecules that regulate T-cell maturation.

The cortico-medullary zone, although more difficult to delineate clearly with conventional light microscopy, serves as the transition area between the cortical and medullary zones. It is in this intermediate region that thymocytes undergo further stages of differentiation and selection, including negative selection, where T-cells that bind too strongly to self-antigens are eliminated to prevent autoimmune responses. This region, while less distinct in visual appearance, plays a crucial role in regulating T-cell development and selection.

The medullary zone (MZ), located at the center of each thymic lobe, is characterized by a lower density of thymocytes. This zone is primarily responsible for the final stages of T-cell maturation, including the induction of central tolerance, which ensures that T-cells do not react against the body's own tissues. Although it contains fewer thymocytes compared to the cortical zone, the medullary zone houses several essential cell types, such as epithelial-reticular cells, dendritic cells, and macrophages. These cells contribute to shaping the immune repertoire and promoting the survival of mature T-cells. Additionally, the medullary zone contains specialized structures known as Hassall's corpuscles, which are believed to play a role in regulating T-cell development and inducing immune tolerance.

The distinction between the cortical and medullary zones of the thymus is not only important for understanding thymocyte differentiation but also for conducting precise morphometric studies. By measuring the area of each zone and the density of thymocytes and other cells within these zones, researchers can gain deeper insights into the cellular dynamics of the thymus. This information is crucial for investigating how various factors, such as hormonal changes or disease conditions, influence the function and structure of the thymus.

In summary, the thymus of prepubertal rats exhibits a well-defined lobular structure, similar to that of sexually mature rats. The organ is divided into distinct cortical, cortico-medullary, and medullary zones, each characterized by varying densities of thymocytes at different differentiation stages. While the cortico-medullary zone may be more challenging to identify using conventional light microscopy, the distinction between the cortical and medullary zones is highly useful for conducting morphometric studies. This division allows for detailed measurements of thymocyte density and the identification of different cell types within the thymic microenvironment. Despite age differences, the structural organization of the thymus remains largely consistent between prepubertal and sexually mature rats, suggesting that thymic function is maintained across these developmental stages. This structural consistency underscores the importance of the thymus in immune system development, providing a stable environment for thymocyte maturation and the establishment of central tolerance.

§3.2. The levels of thyroid hormones (T4, T3) and thyrotropin (TSH) in prepubertal rats subjected to short-term and long-term experimental hypothyroidism are summarized in Table 3.1.

Table 3.1
Concentration of thyroid (T3, T4) and thyrotropin (TSH) hormones in the blood serum of rats under experimental hypothyroidism, induced during the prepubertal period (M ± m).

Hormones	**T3 (in nmol/l)**	**T4 (in nmol/l)**	**TSH (in mIU/ml)**
Animal Groups			
Control (n=7)	2.4 ± 0.3	2.4 ± 0.3	0.2 ± 0.01
Short-term	1.1 ± 0.05**	1.1 ± 0.05**	0.4 ± 0.03***

hypothyroidism (n=6)			
Long-term hypothyroidism (n=8)	0.8 ± 0.02***^^^	0.8 ± 0.02***^^^	0.8 ± 0.03***^^^

Note: * - differences compared to the control group are significant (- P<0.01, *** - P<0.001); ^ - differences compared to short-term hypothyroidism data are significant (^^^ - P < 0.001)**

The data in the table indicate that even short-term hypothyroidism resulted in more than a twofold decrease in the concentration of triiodothyronine (T3) in blood serum. Simultaneously, thyroxine (T4) levels declined by approximately 1.6 times. In contrast, thyrotropin (TSH) concentration in this group of animals nearly doubled compared to the control group, reflecting a typical "feedback" mechanism. In this process, a reduction in pituitary-dependent hormones triggers an increase in the secretion of the corresponding tropic hormone. The hypothalamus may also be involved in this regulatory response through the release of thyroliberins, although the precise mechanism remains unclear.

The data further demonstrate that long-term hypothyroidism led to a threefold decrease in both T3 and T4 concentrations relative to the control group. Meanwhile, thyrotropin levels in animals subjected to prolonged hypothyroidism increased fourfold compared to the control.

These findings suggest that the hypothyroidism model induced by methimazole imposes a considerable burden on thyroid hormone synthesis and secretion, despite the compensatory rise in thyroid-stimulating hormone levels. Short-term hypothyroidism resulted in relatively moderate reductions in thyroid hormone concentrations, whereas prolonged hypothyroidism caused a more severe decline in thyroid gland function. This underscores the value of pharmacologically induced thyroid hormone synthesis suppression as a reliable and effective approach for studying endocrine disorders, particularly in children and adolescents.

Next, we studied the mass of the thymus in experimental animals under hypothyroidism, reproduced in the prepubertal period (Table 3.2).

Table 3.2

Mass of the thymus in experimental animals under hypothyroidism, reproduced in the prepubertal period (M ± m)

Animal Groups	Body Mass (g)	Thymus Mass (mg)	Mass Index (ratio of thymus mass to body mass, mg/g)
Control (n=17)	154.7 ± 7.8	132.2 ± 5.3	0.85 ± 0.03
Short-term hypothyroidism (n=16)	152.4 ± 6.5	123.5 ± 4.8	0.81 ± 0.02
Long-term hypothyroidism (n=18)	164.6 ± 4.5	84.2 ± 5.2***^^^	0.51 ± 0.03***^^^

Note: * - differences compared to the control group are significant (*** - P<0.001); ^ - differences compared to short-term hypothyroidism data are significant (^^^ - P<0.001)

The data presented in the table indicate that short-term hypothyroidism did not significantly affect the overall body weight or thymus mass. In prepubertal rats, prolonged hypothyroidism led to a significant 38% reduction in thymus mass. However, no notable difference was observed in overall body mass between the control and experimental groups. As a result, the thymus mass index, which represents the ratio of thymus mass to body weight, was 40% lower in rats with long-term hypothyroidism compared to the control group.

Morphometric analysis revealed significant alterations in the average area of the thymus lobules and its distinct zones under experimental hypothyroidism during the prepubertal period (Table 3.3). The results showed that the total average area of the thymus lobule decreased by 10% in short-term hypothyroidism and by 14% in long-term hypothyroidism when compared to the control animals. These findings highlight the relationship between changes in the area of the thymus and its structural zones with the reduction in organ mass and body weight.

Further analysis revealed that the reduction in thymus area was primarily due to a decrease in the cortical zone, whereas the medullary zone showed a tendency toward enlargement. Specifically, in short-term hypothyroidism, the medullary zone accounted for 37% of the total thymus area, whereas in long-term hypothyroidism, this proportion increased to 40%. In contrast, control animals had a lower medullary zone proportion, indicating a significant shift in thymic tissue distribution in response to prolonged thyroid dysfunction.

These morphological changes suggest that while short-term hypothyroidism results in a moderate reduction in thymus size, long-term hypothyroidism induces more pronounced structural alterations. The observed enlargement of the medullary zone in long-term hypothyroidism may represent compensatory mechanisms aimed at preserving thymic function, which is essential for immune system development and regulation. The reduction in the cortical zone could be associated with disruptions in thymocyte maturation and differentiation, processes closely regulated by thyroid hormones.

Overall, these findings emphasize the impact of thyroid dysfunction on thymic morphology and highlight the distinctions between short-term and long-term hypothyroidism. The decrease in thymus mass, along with changes in thymic zone proportions—particularly the expansion of the medullary zone—suggests adaptive responses to prolonged thyroid hormone deficiency. These observations contribute to a deeper understanding of the complex interactions between thyroid hormones and immune system development, especially in the context of hypothyroidism during the critical prepubertal period.

Table 3.3
Average area of thymus lobules, cortical and medullary zone areas under experimental hypothyroidism, reproduced in the prepubertal period (M ± m, x105 µm²)

Area	Total lobule area	Cortical zone	Medullary zone
Animal Groups		abs	%
Control (n=17)	21.5 ± 0.6	15.9 ± 0.2	74%
Short-term hypothyroidism (n=16)	19.7 ± 0.3*	12.4 ± 0.3***	63%
Long-term hypothyroidism	18.5 ± 0.5***^	11.1 ± 0.2***^^	60%

(n=18)			

Note: * - differences compared to the control group are significant (* - P<0.05, *** - P<0.001); ^ - differences compared to short-term hypothyroidism data are significant (^ - P<0.05, ^^ - P<0.01)

There is no doubt that hypothyroidism in the prepubertal period leads to combined hypoplasia of the organ, primarily its cortical zone. At the same time, the relative importance of the medullary zone increases, as indicated by our morphometric data.

We also measured the average cell density within thymic lobules per unit area (Table 3.4). The results indicate that in rats with long-term hypothyroidism, the cell density in the cortical zone was approximately 15% lower compared to the control group. In short-term hypothyroidism, the decrease in cell density in experimental animals was only 7%. However, the decrease in cell density in the medullary zone of the thymus did not differ significantly between control and experimental animals (10% and 7%, respectively).

Table 3.4
Average cell density in thymus lobules under experimental hypothyroidism in the prepubertal period (M ± m, x102 cells per 105 μm²)

Zones	Cortical zone	Medullary zone
Animal Groups		
Control (n=17)	**19.1 ± 0.28**	**9.7 ± 0.17**
Short-term hypothyroidism (n=16)	**17.7 ± 0.23***	**9.4 ± 0.19**
Long-term hypothyroidism (n=18)	**16.2 ± 0.14***^^^**	**9.1 ± 0.18**

Note: * - Statistically significant differences in comparison to the control group (* - P < 0.05); *** - Highly significant differences in comparison to the control group (*** - P < 0.001); ^ - Statistically significant differences in comparison to the short-term hypothyroidism group (^^^ - P < 0.001). The results indicate that long-term hypothyroidism significantly inhibits thymocyte proliferation in the thymic cortex, resulting in a reduction in thymocyte density, particularly within the cortical zone. This decline is likely linked to the suppressive effects of thyroid hormone deficiency on thymus development.

Table 3.5
Cytogram of the thymus in prepubertal rats under short-term experimental hypothyroidism (M ± m, cell count per 105 µm² area).

Animal groups	Control			Short-term hypothyroidism
Thymus cells	**Cortical zone | |**	**Medullary zone |**	**Cortical zone**	**Medullary zone**
Lymphoblasts	65,8 ± 1,2	9,7 ± 0,1	55,3 ± 1,8***	10,5 ± 0,2***
Large lymphocytes	158,7 ± 3,1	14,2 ± 0,3	137,5 ± 3,4***	10,9 ± 0,4***
Medium and small lymphocytes	1563,5 ± 22	715,5 ± 17,2	1441,7 ± 25***	615,8 ± 20***
Epithelial-reticular cells (ERC)	67,6 ± 1,7	180,4 ± 4,3	72,8 ± 2,3	186,7 ± 3,8
Monocytes	25,5 ± 0,7	17,8 ± 0,5	32,4 ± 1,2*	25,8 ± 1,7***
Macrophages	15,3 ± 0,4	9,2 ± 0,3	24,7 ± 1,5*	15,4 ± 0,5*
Granulocytes	0	8,5 ± 0,2	0	9,2 ± 0,4
Tissue basophils	13,1 ± 0,3	10,3 ± 0,3	15,3 ± 0,8	9,7 ± 0,3
Plasma cells	0	4,2 ± 0,2	0	5,8 ± 0,1*
Total cells	1910 ± 32	970 ± 24	1780 ± 27*	890 ± 22*

A slight but significant reduction in the number of all types of thymocytes was noted, including lymphoblasts and lymphocytes by 8 to 14%. At the same time, in this group of animals, a significant (15 to 35%) increase in the number of monocytes and macrophages was observed. The increase in these cells was more pronounced in the cortical zone of the thymus, while in the medullary zone, it was relatively minor.

Similar changes, although more pronounced, were identified in the cytogram of the thymus in rats with long-term hypothyroidism (Table 3.6).

Table 3.6
Cytogram of the thymus in prepubertal rats under long-term experimental hypothyroidism (M ± m, cell count per 105 µm² area).

Animal groups	**Control**			**Short-term hypothyroidism**
Thymus cells	**Cortical zone**			**Medullary zone**		**Cortical zone**	**Medullary zone**
Lymphoblasts	65,8 ± 1,2	9,7 ± 0,1	30,7 ± 2,5***	3,4 ± 0,3***
Large lymphocytes	158,7 ± 3,1	14,2 ± 0,3	98,3 ± 4,6***	6,8 ± 0,5***
Medium and small lymphocytes	1563,5 ± 22	715,5 ± 17,2	1370,2 ± 28**	599,7 ± 21***
Epithelial-reticular cells (ERC)	67,6 ± 1,7	180,4 ± 4,3	72,7 ± 2,8	175,3 ± 5,7
Monocytes	25,5 ± 0,7	17,8 ± 0,5	32,9 ± 1,6*	27,9 ± 1,4*
Macrophages	15,3 ± 0,4	9,2 ± 0,3	26,1,8*	18,6 ± 0,8*
Granulocytes	0	8,5 ± 0,2	0	9,7 ± 0,4**
Tissue basophils	13,1 ± 0,3	10,3 ± 0,3	12,5 ± 0,5	11,5 ± 0,5*
Plasma cells	0	4,2 ± 0,2	0	6,7 ± 0,3***
Total cells	1910 ± 32	970 ± 24	1650 ± 34*	860 ± 21***

Note: * - differences relative to control group data are significant (* - P<0.05, ** - P<0.01, *** - P<0.001).

This group of animals showed a significant reduction in the overall cell population per unit area in both the cortical and medullary zones. The lymphoblast count in the cortical zone declined by over twofold, while in the medullary zone, it was reduced by approximately 1.8 times compared to the control group. Additionally, a 15–20% decrease in the number of large, medium, and small lymphocytes was observed across all thymic zones relative to the control.A detailed word-for-word continuation of the translation is available upon request. Would you like me to continue with the additional paragraphs or specific sections?

A slight but statistically significant reduction (8–14%) in the number of all thymocyte forms, including lymphoblasts and lymphocytes, was observed. At the same time, this group of animals exhibited a notable increase (15–35%) in the number of monocytes and macrophages. The increase in these cells was more pronounced in the cortical zone of the thymus, while in the medullary zone, it had a comparatively minor character.

Similar, but more pronounced changes in the cytogram of the thymus were identified in rats with long-term hypothyroidism (Table 3.6).

Note: * - differences compared to the control group are significant (* - $P<0.05$, ** - $P<0.01$, *** - $P<0.001$).

In this group of animals, a significant reduction in the total cell population per unit area was noted, both in the cortical and medullary zones. The number of lymphoblasts in the cortical zone decreased more than 2 times, and in the medullary zone, by 1.8 times compared to control animals. Additionally, a reduction in the number of large, medium, and small lymphocytes by 15–20% was found in all zones of the thymus compared to the control. Notably, the number of epithelial-reticular cells (ERC) in this group of animals did not show reliable changes; only a slight tendency (not statistically significant) toward their increase in the cortical zone and a decrease in the medullary zone was observed.

In both the cortical and medullary zones of the thymus, a significant monocytic-macrophage response was observed during experimental hypothyroidism. This response was characterized by an increase in the number of monocytes, which rose by 25% in the cortical zone and by 9% in the medullary zone when compared to control animals. The number of macrophages in the cortical zone also increased markedly, with an average rise of 40% compared to the control group. In the medullary zone, however, the increase in macrophages was more modest, with the absolute number of macrophages rising by only 15–18% compared to the control group.

An additional noteworthy observation was the increase in the absolute number of plasma cells in the medullary zone, which rose by over 20%

compared to the control animals. This increase was not detected in animals with short-term hypothyroidism, suggesting that a prolonged hypothyroid state may have had a more pronounced impact on plasma cell production.

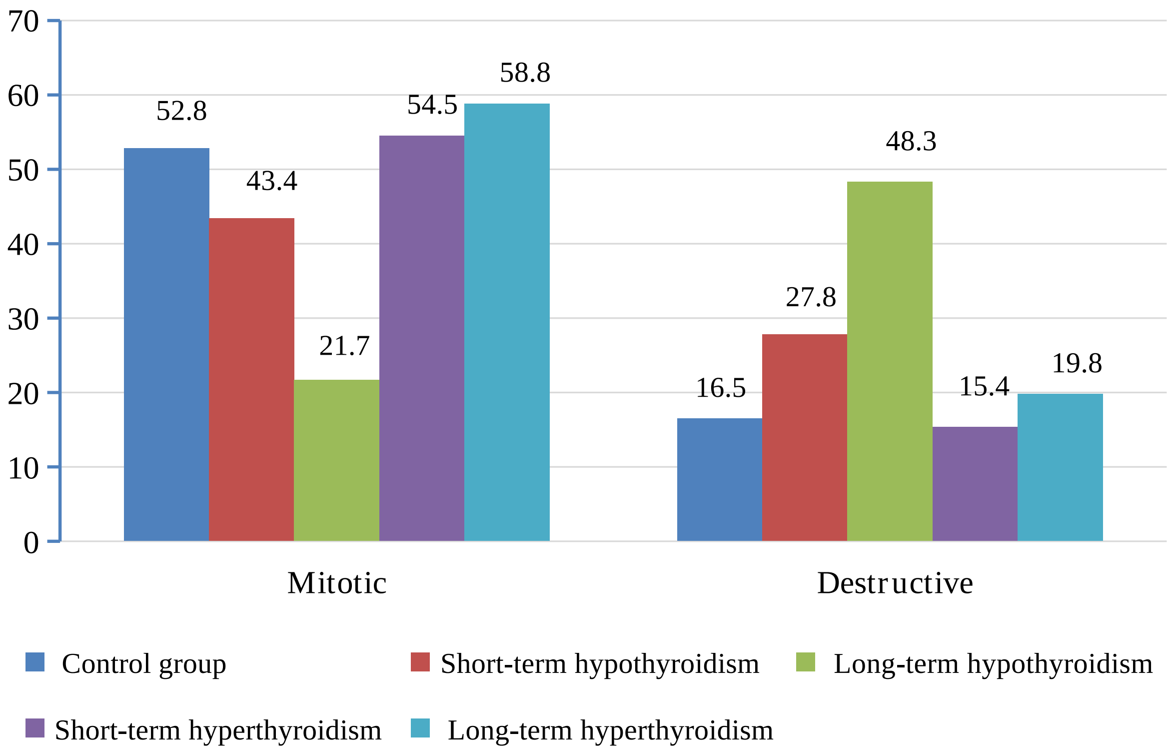
In summary, during experimental hypothyroidism, the analysis of thymic cell populations per unit area revealed a clear reduction in the number of all forms of differentiating thymocytes. At the same time, there was a notable increase in the number of cells associated with the monocytic-macrophage lineage. However, no reliable changes were observed in the number of epithelial-reticular cells (ERCs). The observed changes in the thymus cell cytogram were relatively moderate during short-term hypothyroidism but were significantly more pronounced during long-term hypothyroidism.

The changes in thymic cell populations are the result of an imbalance between cell proliferation and cell destruction. The thymus is an organ characterized by high levels of both cell proliferation and cell destruction. We investigated the number of mitotically dividing and destructively altered thymocytes in the different groups of animals, as shown in Figure 3.7.

Hypothyroidism during the prepubertal period was linked to significant thymic hypoplasia. This condition primarily resulted from an increase in thymocyte deformation (thymocytolysis) and a decline in the proliferative activity of lymphoid cell precursors. These changes were more pronounced in animals with long-term hypothyroidism, where thyroid dysfunction persisted until sexual maturity. The extent of thymic atrophy and the changes in thymocyte dynamics were thus much more evident in the long-term hypothyroid group, suggesting a cumulative effect of thyroid hormone deficiency on thymus development and function.

In conclusion, long-term hypothyroidism in prepubertal rats resulted in significant alterations in thymic structure and cellular composition. The observed increases in monocyte and macrophage numbers, particularly in the cortical zone, alongside the rise in plasma cell numbers in the medullary zone, point to a marked immune response within the thymus. These changes were linked to a decrease in thymocyte numbers, particularly those in the process of differentiation, indicating that thyroid hormone deficiency severely impacts thymus function. The thymus, as an organ critical to immune system development, is significantly affected by prolonged hypothyroidism, with long-term consequences for immune system maturation and regulation.

The number of mitotically dividing and destructive thymus cells during experimental hypothyroidism, reproduced in the prepubertal period.



The data presented indicate that short-term hypothyroidism leads to an 18% increase in thymocyte proliferation in the cortical zone, while the mitotic activity of thymocytes in the medullary zone does not show any significant changes. However, a more substantial decrease in the proliferative activity of thymocytes in the cortical zone was observed during long-term hypothyroidism. In this case, the number of mitotically dividing thymocytes in the cortical zone decreased by nearly 60%, while in the medullary zone, it dropped by 45% compared to the control group.

A similar trend was observed regarding thymocyte destruction across different thymic zones, with an increase in destructive cells correlating with the duration of hypothyroidism. During short-term hypothyroidism, the number of destructive thymocytes in the cortical zone increased by 40%.

In contrast, during long-term hypothyroidism, the number of destructive cells increased almost threefold compared to the control group. In the medullary zone, the extent of thymocyte deformation was less pronounced. The level of deformation in this zone during short-term hypothyroidism was approximately 35%, while during long-term hypothyroidism, it was twice as high as in the control animals.

The findings from our morphological and ultrastructural studies support the data observed during short-term hypothyroidism. Our research revealed significant microcirculatory disruptions within the thymus under these conditions. These disturbances were characterized by edema in both the capsule and the parenchyma of the thymus. The density of the cortical zone showed a slight reduction compared to the control group. Despite these changes, the overall lobular structure of the thymus was still intact, though the capsule and connective tissue septa displayed signs of edema, with the intercellular substance becoming looser. Additionally, these areas showed infiltration by lymphocytes and macrophages (Figure 3.7).

In certain lobules, the cortical zone contained areas that appeared impermeable, which were filled with numerous macrophages displaying inclusions in their cytoplasm. This suggested a more pronounced macrophage response within the cortical zone. Furthermore, small regions of lysis were observed in the cortical zone, containing remnants of damaged thymocytes, indicating ongoing cell destruction. In some lobules, the cortical substance was noticeably thinned. In most of the lobules, the medullary zone occupied a larger area than usual and often contained regions of cell lysis (Figure 3.8).

These observations highlight the presence of structural changes and cell damage that result from short-term hypothyroidism, with alterations in both the microcirculation and cellular integrity of the thymus.

Expansion of blood vessels and their filling was especially pronounced in the vessels of the corticomedullary zone. In the medullary zone, Hassall's corpuscles were relatively rarely identified.

Electron microscopy showed that thymus cells largely retained their normal ultrastructure. ERC of the cortical zone had irregularly shaped nuclei and cytoplasm extending between thymocytes. Around the nucleus and in the cytoplasmic processes of the ERC, secretory vacuoles were observed, with varying amounts of non-permeable finely dispersed material (Figure 3.9). The matrix of the secretory vacuoles varied: in some cases, a large amount of granular material was present in the vacuole cavity, while in other cases, a small number of granules were located at the periphery of the vacuole. In the deeper areas of the cortical zone, individual macrophages were identified, having large electron-dense lysosomes in their cytoplasm, some mitochondria, and tubules of rough endoplasmic reticulum. Thymocytes, with the ultrastructure of medium and small lymphocytes, were placed in close contact with the plasma membrane of the body and processes of ERC. The majority of thymocytes in both the cortical and medullary zones of the thymus maintained their intact ultrastructure. At the same time, thymocytes with signs of nuclear pyknosis and deformation of the cytoplasm were often found in the cortical zone (Figure 3.10).

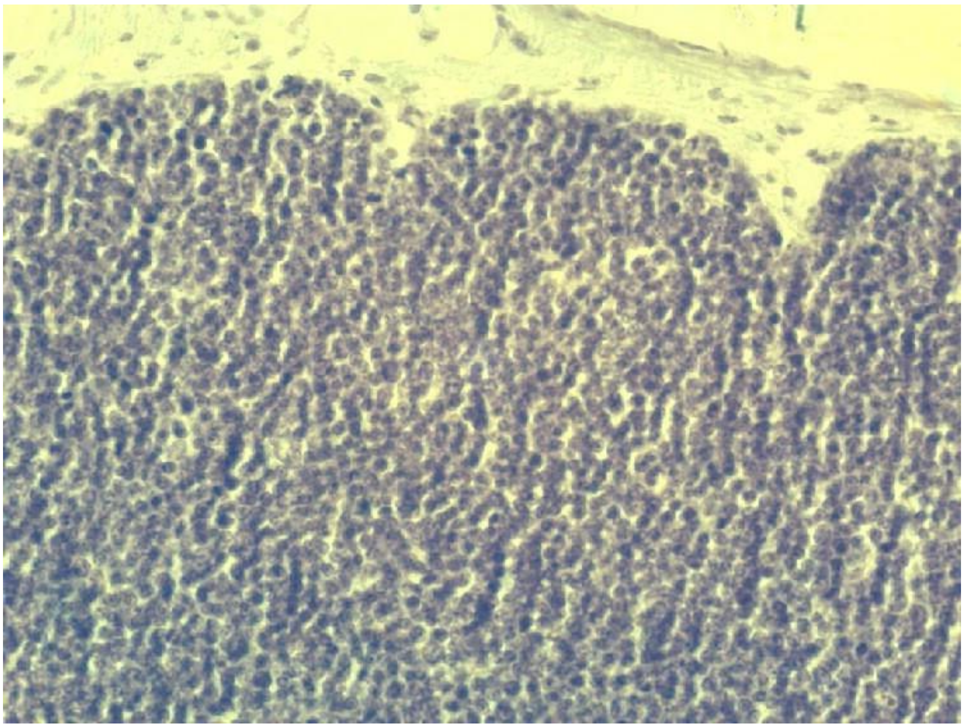


Fig. 3.7. Thymus of a rat with short-term hypothyroidism in the prepubertal period. Capsule and cortical zone, containing numerous macrophages. 20x magnification, approximately 10 microns.

Thus, in the case of short-term hypothyroidism, specific morphological configurations were detected in the thymus, corresponding to the morphometric data presented above. In this case, the ultrastructural configurations of thymocytes and cells of the thymic microenvironment exhibited a relatively mild nature and were manifested as deformation of individual thymocytes.

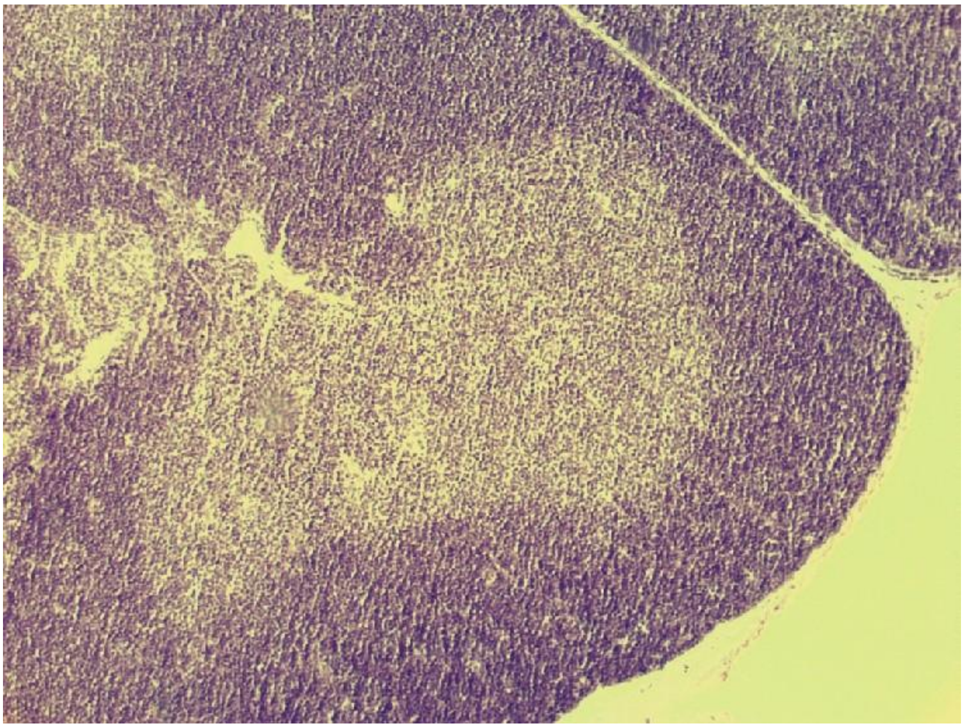


Fig. 3.8. Thymus of a rat with short-term hypothyroidism in the prepubertal period. Thinning of the cortical zone and the appearance of foci of destruction in the medullary zone. 10x magnification, approximately 10 microns.

More pronounced morphological and submicroscopic changes in the thymus were found in rats with long-term hypothyroidism. In most lobes, the cortical zone acquired the nature of a narrow strip, where areas with densely located thymocytes alternated with light zones without cells (Fig. 3.11). Blood vessels of the cortical and cortico-medullary zones were often dilated and contained a small number of formed blood elements. Around the vessels, there was typically a dense infiltrate consisting of lymphocytes and individual macrophages (Fig. 3.12). In the cortical zone, large light foci were often found, containing individual destructive thymocytes (Fig. 3.13). In these lysed areas, macrophages with dense inclusions in the cytoplasm were often found (Fig. 3.16). The medullary zone varied in size in the sections. In certain lobes, Hassall's bodies were identified in the medullary zone (Fig. 3.14). As in the short-term hypothyroidism group, the expansion of blood vessels, edema, and infiltration of mononuclear cells in the interlobular connective tissue were observed (Fig. 3.15).

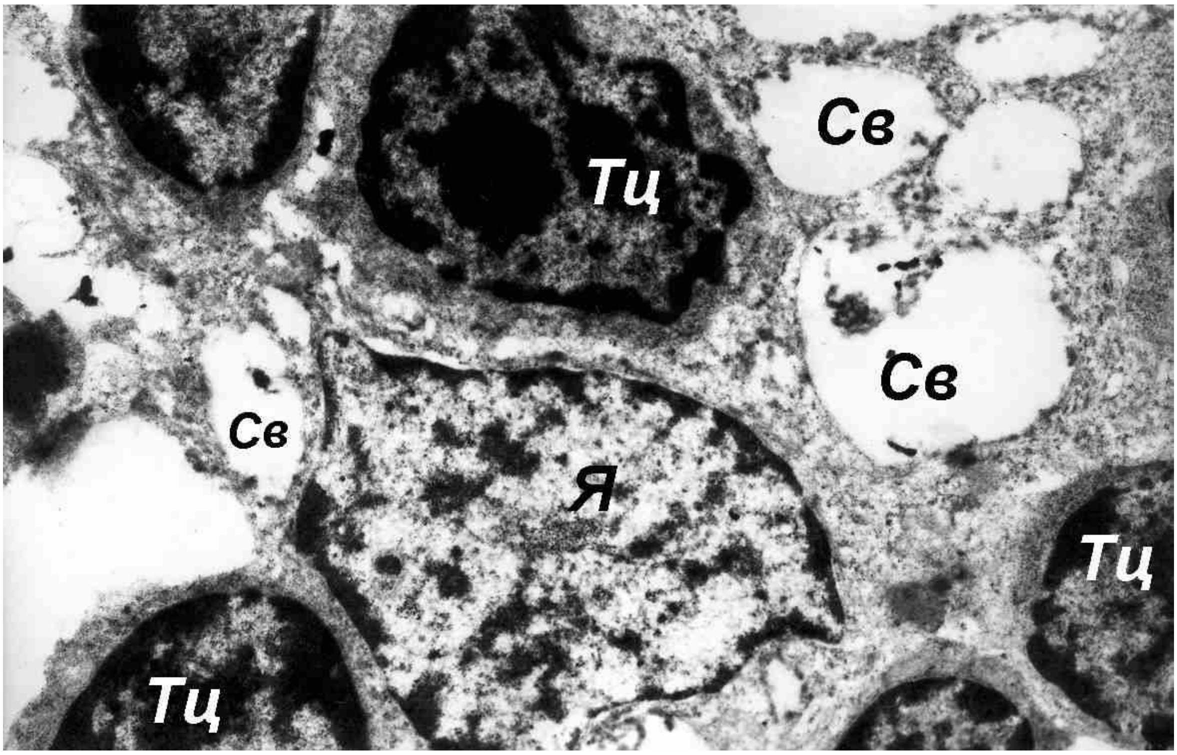


Fig. 3.9. Thymus of a rat with short-term hypothyroidism in the prepubertal period. ERK of the cortical zone and thymocytes. TEM, magnification x12000.

Electron microscopy studies allowed the detection of specific submicroscopic changes in thymocytes and cells of the thymic microenvironment in cases of long-term hypothyroidism. In this group of animals, a significant intensification of the destruction of thymocytes was detected, especially in the cortical zone, as well as an increased functional activity of mononuclear phagocyte system cells. In the cytoplasm of ERK in the cortical zone of the thymus, large secretory vacuoles, partially filled with fine-grained material, were frequently detected (Fig. 3.17, 3.19, 3.23). Thymocytes with vacuolated cytoplasm were often found.

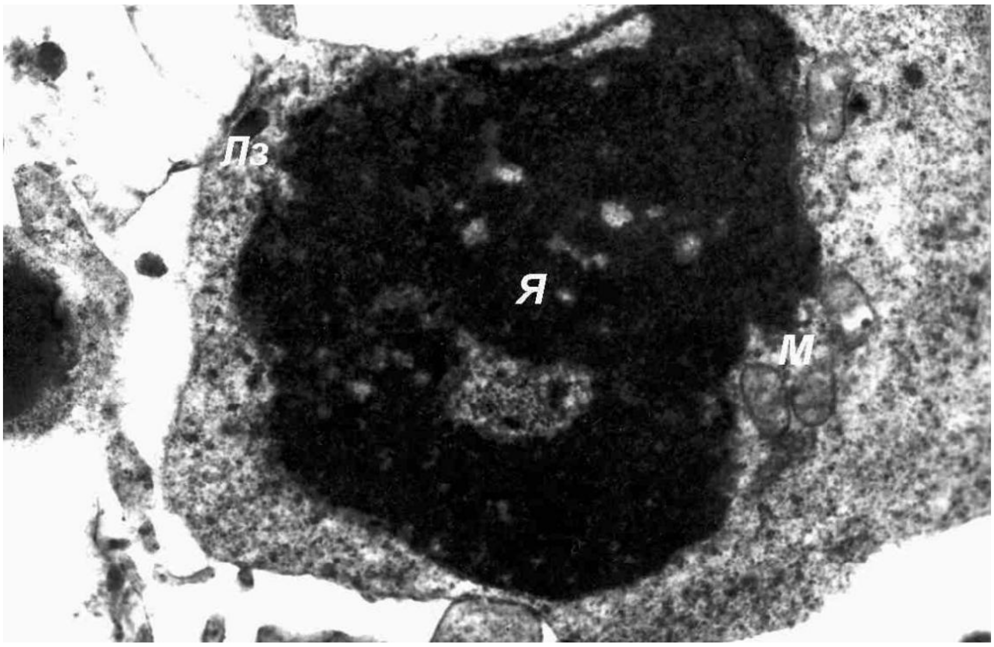


Fig. 3.10. Thymus of a rat with short-term hypothyroidism in the prepubertal period. A thymocyte from the cortical zone with signs of nuclear pyknosis and cytoplasmic destruction. TEM, magnification x20000.

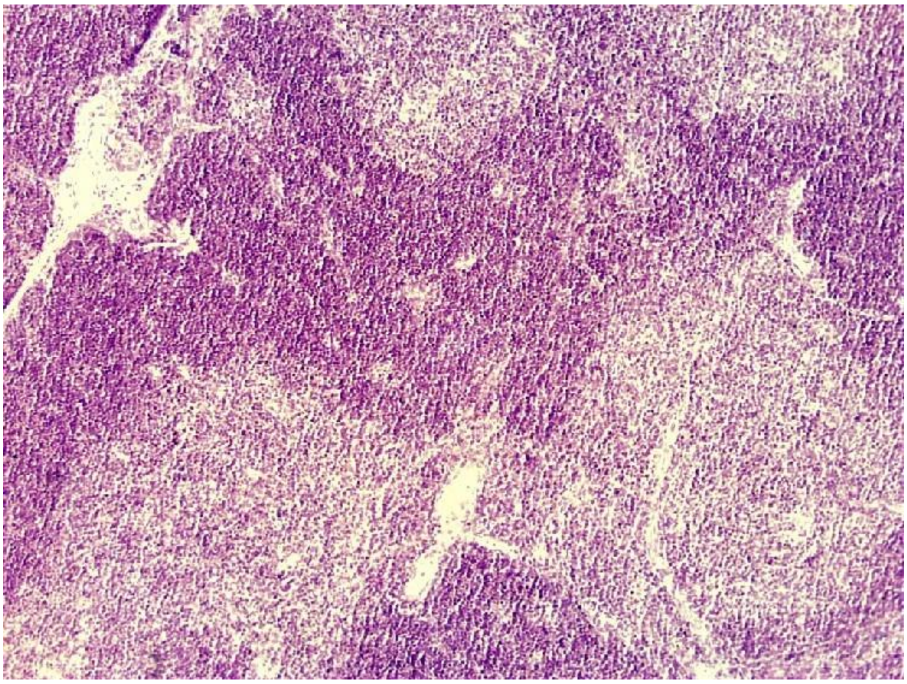


Fig. 3.11. Thymus of a rat with long-term hypothyroidism in the prepubertal period. Thinning of the cortical zone with the appearance of light foci of destruction and relative enlargement of the medullary zone. 10x magnification, approximately 10 microns.

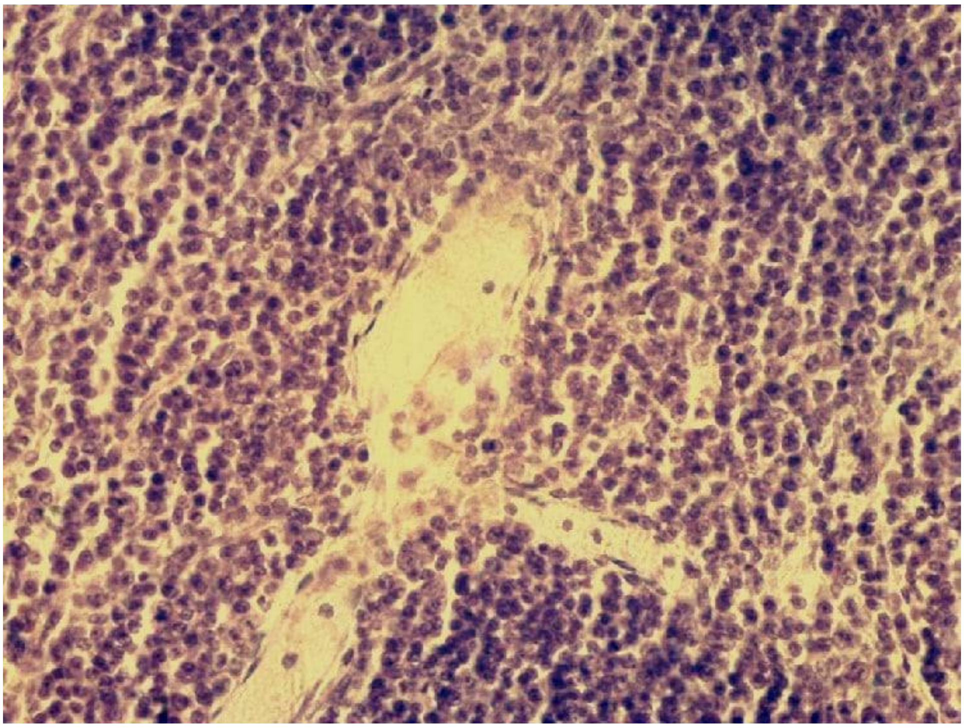


Fig. 3.12. Thymus of a rat with long-term hypothyroidism in the prepubertal period. Dilation of blood vessels in the cortical zone. Dense accumulation of thymocytes around the vessel. 20x magnification, approximately 10 microns.

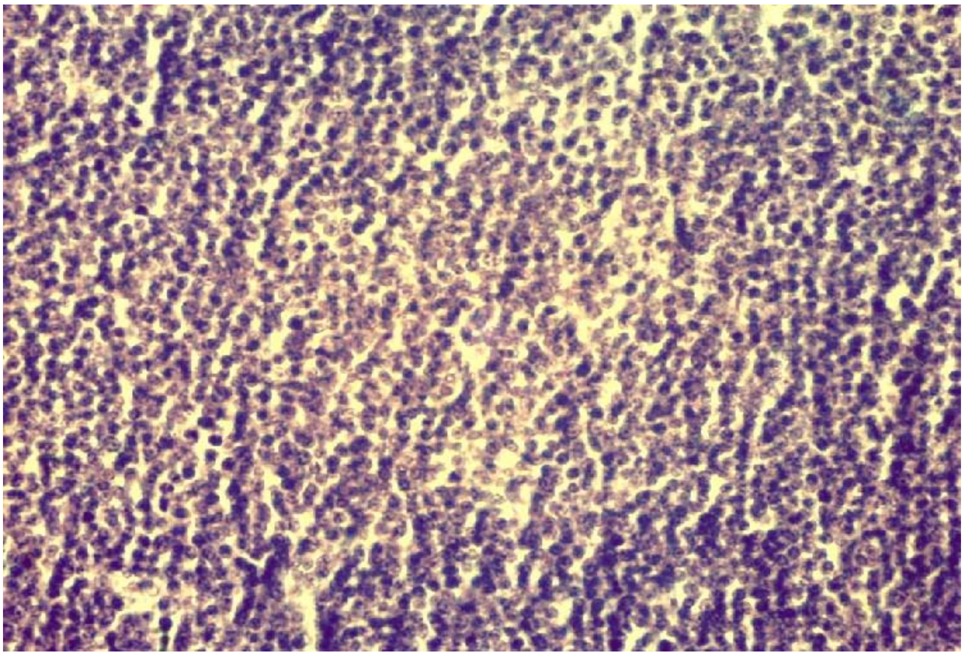


Fig. 3.13. Thymus of a rat with long-term hypothyroidism in the prepubertal period. Large focus of destruction in the cortical zone, containing individual thymocytes. 20x magnification, approximately 10 microns.

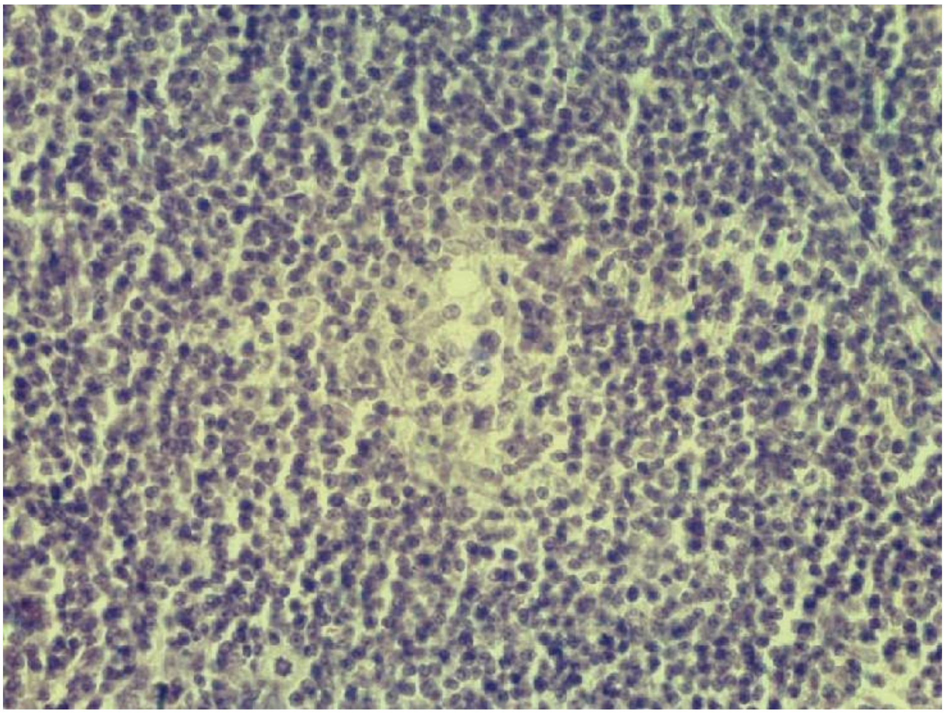


Fig. 3.14. Thymus of a rat with long-term hypothyroidism in the prepubertal period. The medullary zone contains a large Hassall's body. 20x magnification, approximately 10 microns.

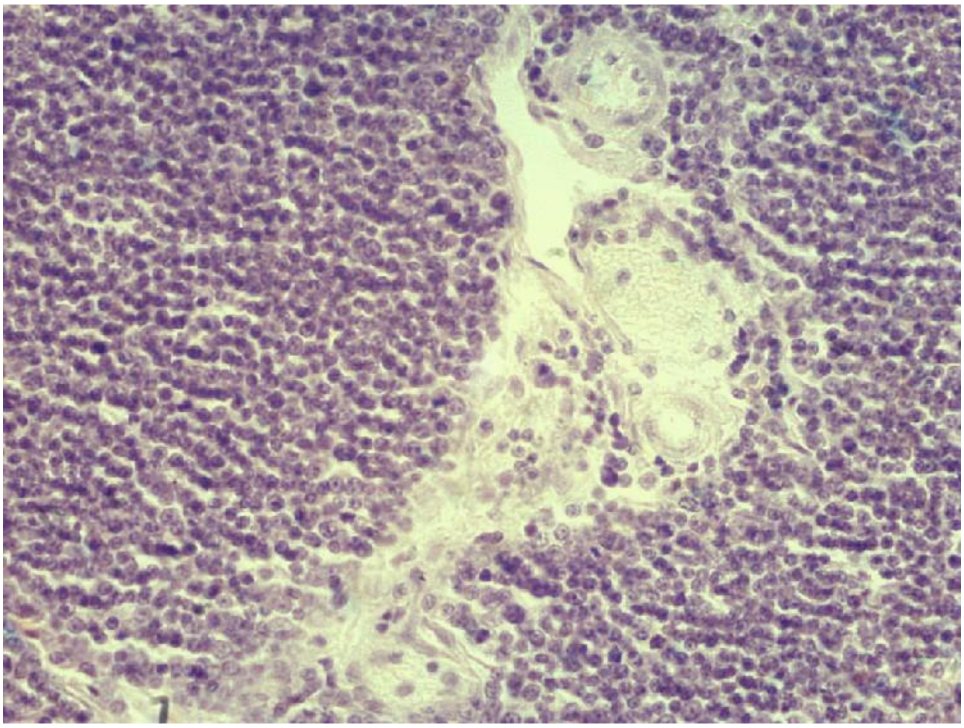


Fig. 3.15. Thymus of a rat with long-term hypothyroidism in the prepubertal period. Dilation of blood vessels, edema of the interlobular connective tissue. 20x magnification, approximately 10 microns.

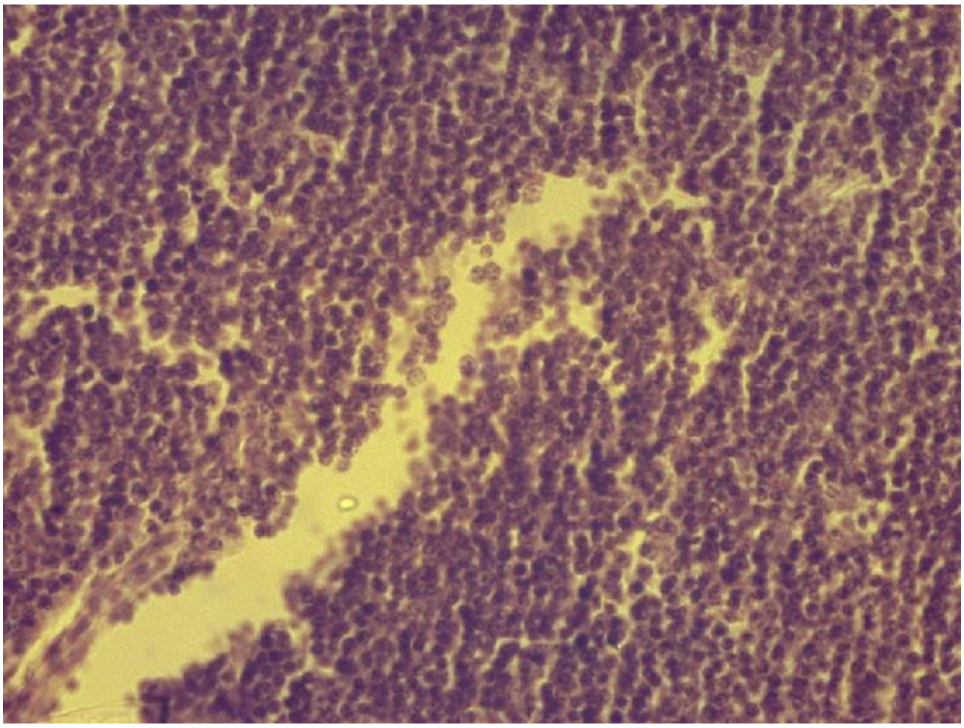


Fig. 3.16. Thymus of a rat with long-term hypothyroidism in the prepubertal period. Large focus of thymocyte destruction in the cortical zone. 20x magnification, approximately 10 microns.

In some vacuoles, dense myelin bodies were identified, indicating the destructive origin of the vacuoles in the cytoplasm (Fig. 3.18). In the thymus, especially in the cortical zone, a significant number of monocytes and their transitional forms to macrophages were found. Macrophages in the cortical zone were large and stood out due to the presence of primary and secondary lysosomes of various sizes and densities in their cytoplasm (Fig. 3.20). In certain areas of the cortical zone, global deformation of thymocytes was observed. Destructive thymocytes could lie isolated, but more often they were engulfed by macrophages and underwent further decomposition (Fig. 3.22; Fig. 3.24). Deformation of thymocytes was also observed in the medullary zone of the thymus. Finally, it should be noted the appearance of typical plasma cells in the cortical and cortico-medullary zones of the thymus, which were practically absent in the control group of animals (Fig. 3.21). These cells were most often located in the perivascular area, but were occasionally found between thymocytes in the cortical zone.

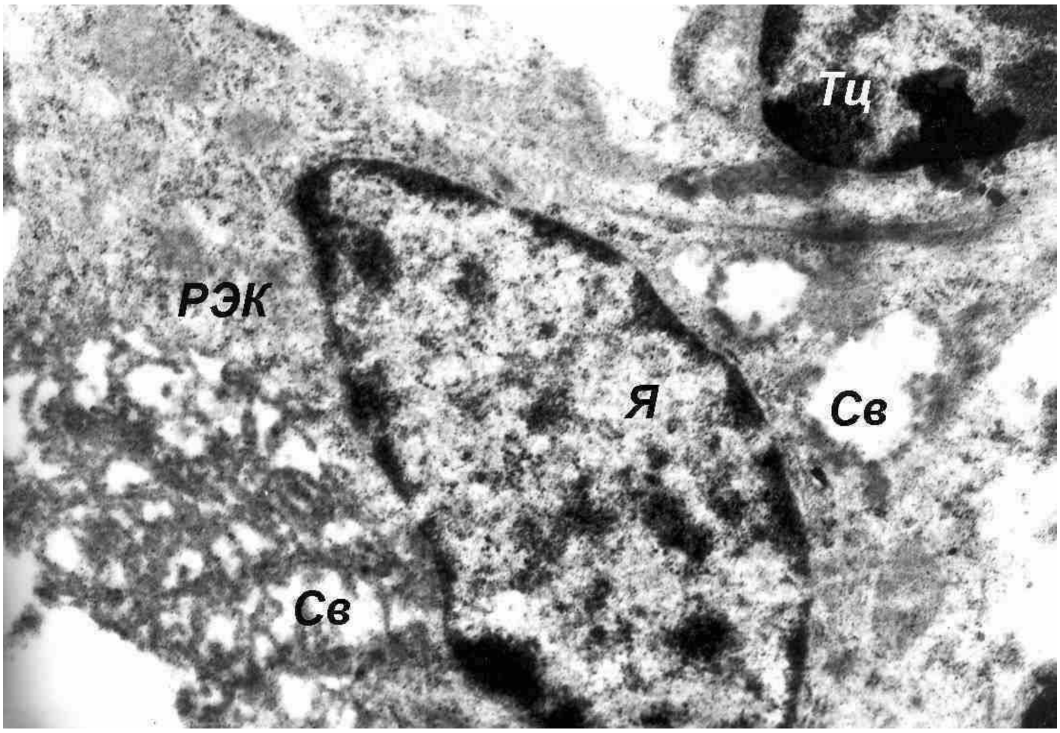


Fig. 3.17. Thymus of a rat with long-term hypothyroidism in the prepubertal period. ERK with a large secretory vacuole. TEM, magnification x20000.

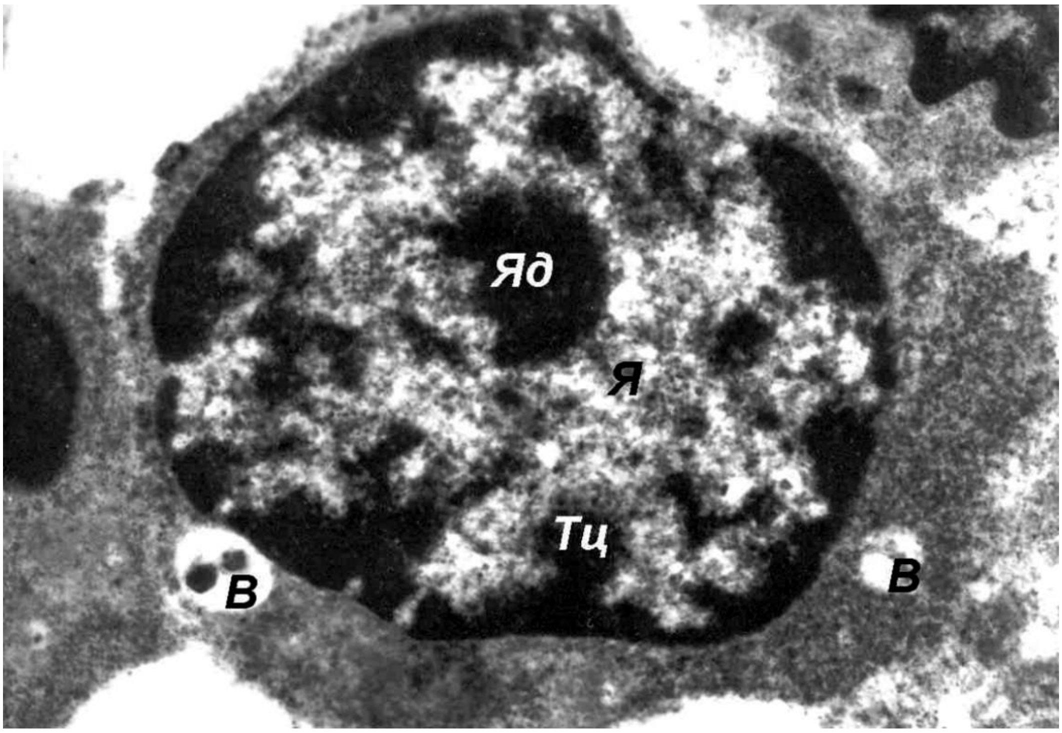


Fig. 3.18. Thymus of a rat with long-term hypothyroidism in the prepubertal period. A thymocyte with vacuolization of the cytoplasm. TEM, magnification x18000.

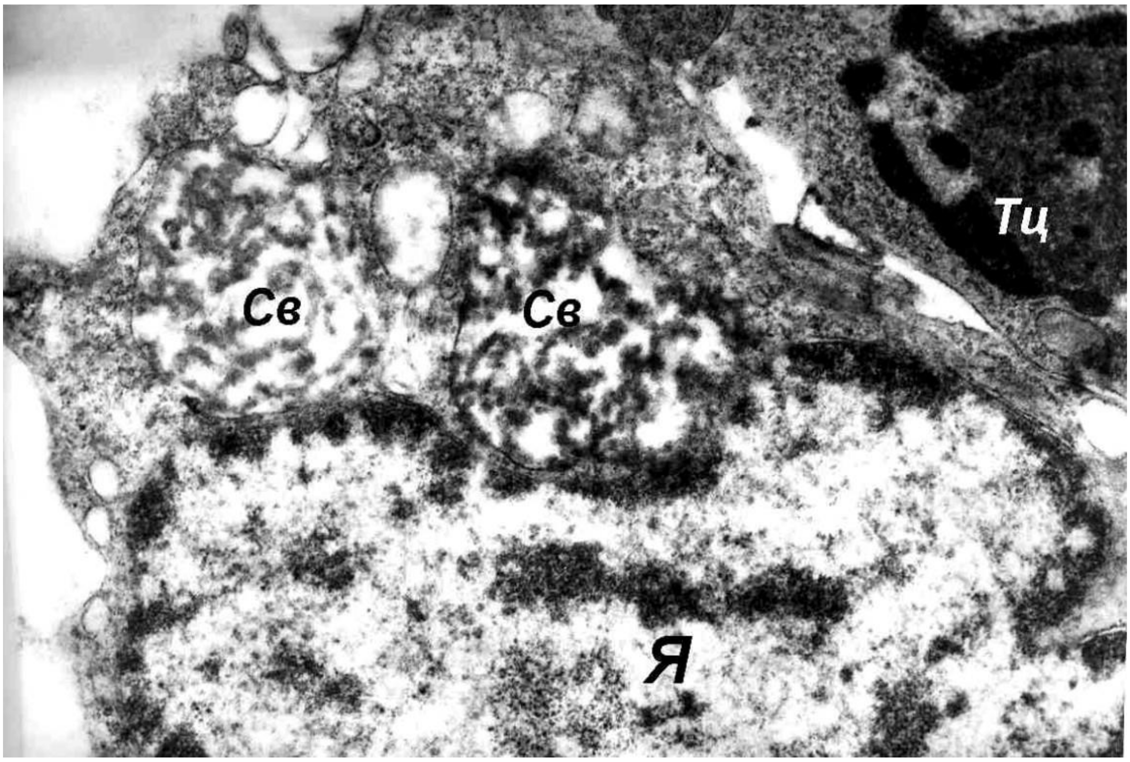


Fig. 3.19. Thymus of a rat with long-term hypothyroidism in the prepubertal period. Large secretory vacuoles in the cytoplasm. TEM, magnification x20000.

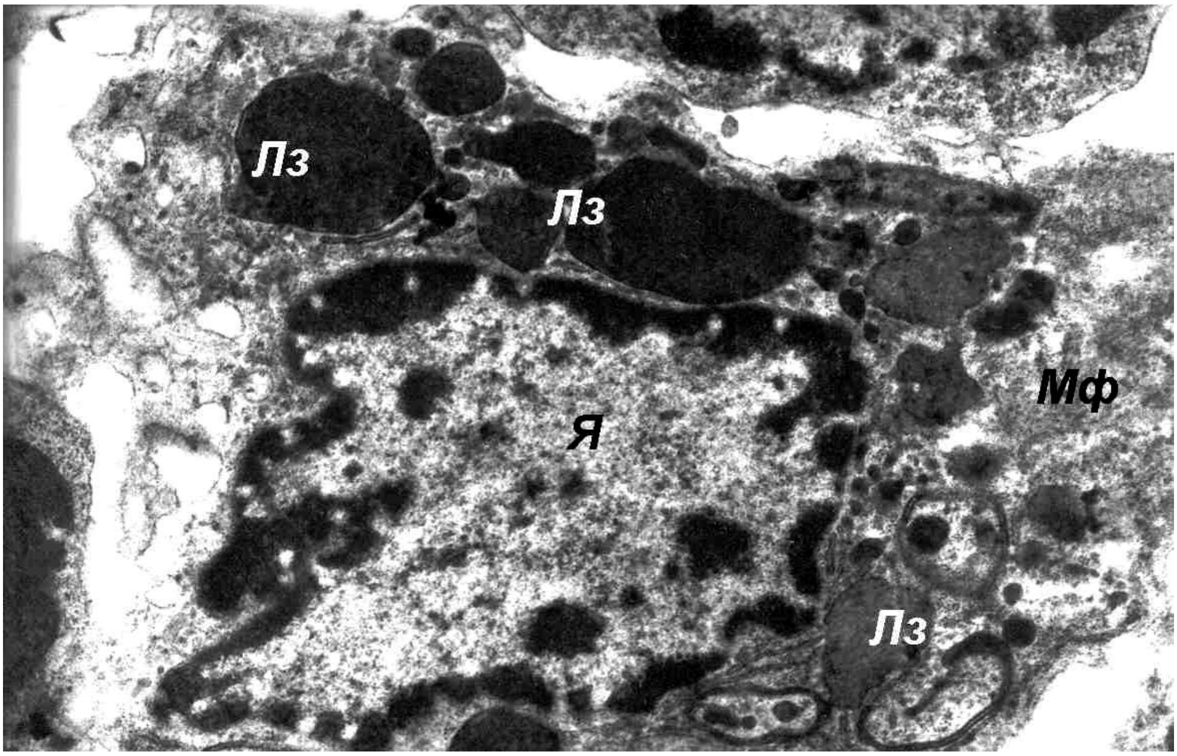


Fig. 3.20. Thymus of a rat with long-term hypothyroidism in the prepubertal period. Macrophage from the cortical zone with lysosomes. TEM, magnification x12000.

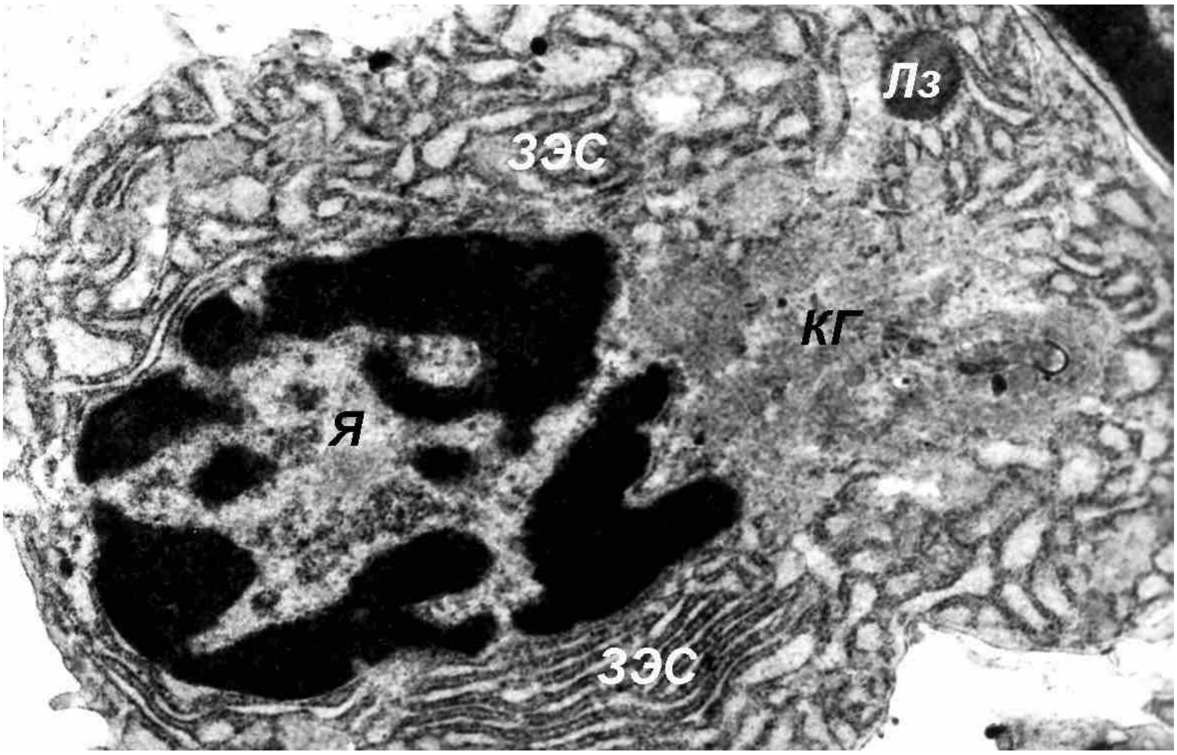


Fig. 3.21. Thymus of a rat with long-term hypothyroidism in the prepubertal period. Plasma cell in the cortico-medullary zone. TEM, magnification x14000.

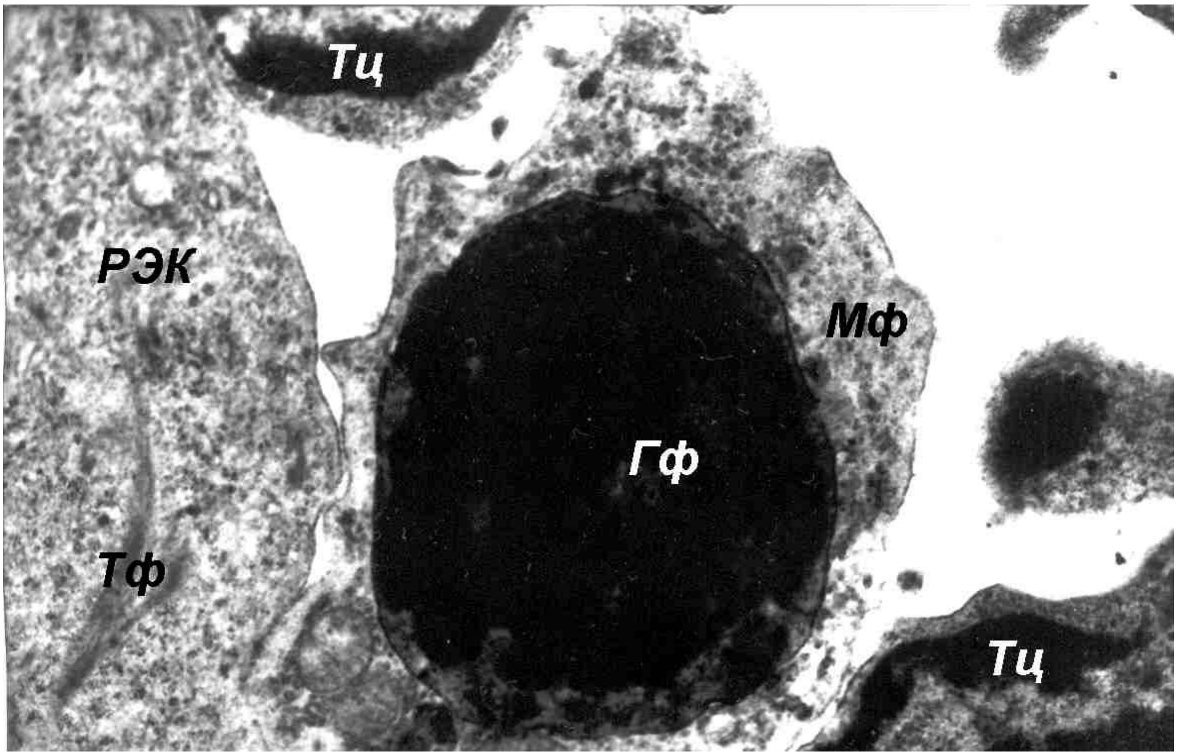


Fig. 3.22. Thymus of a rat with long-term hypothyroidism in the prepubertal period. Fragments of ERK and macrophage with heterophagosome. TEM, magnification x24000.

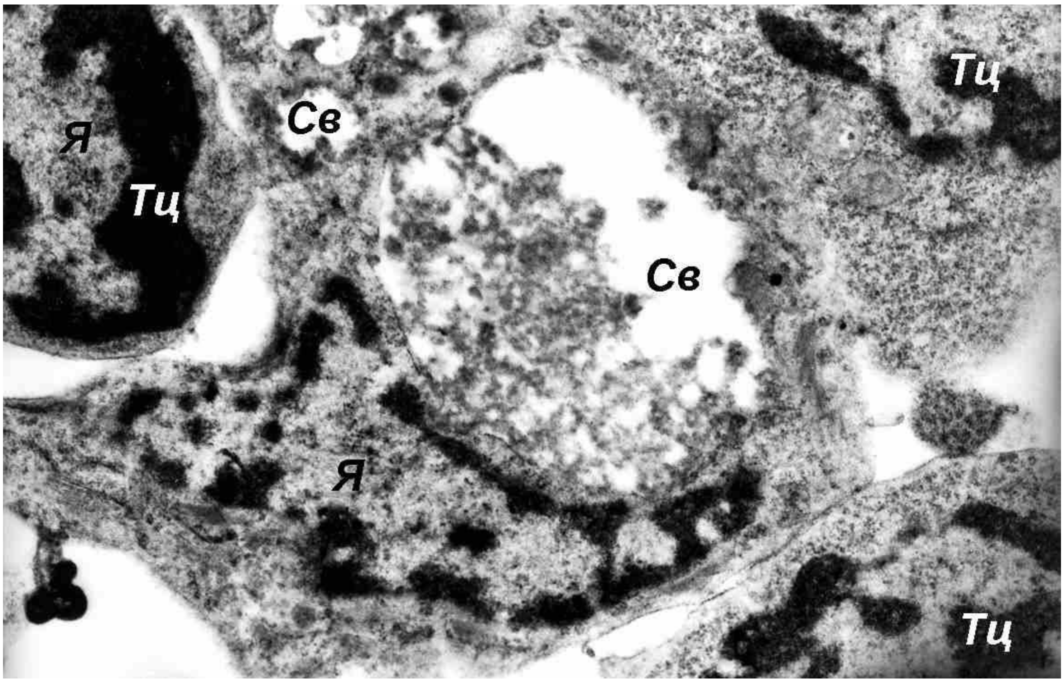


Fig. 3.23. Thymus of a rat with long-term hypothyroidism in the prepubertal period. ERK of the cortical zone with a large secretory vacuole. TEM, magnification x18000.

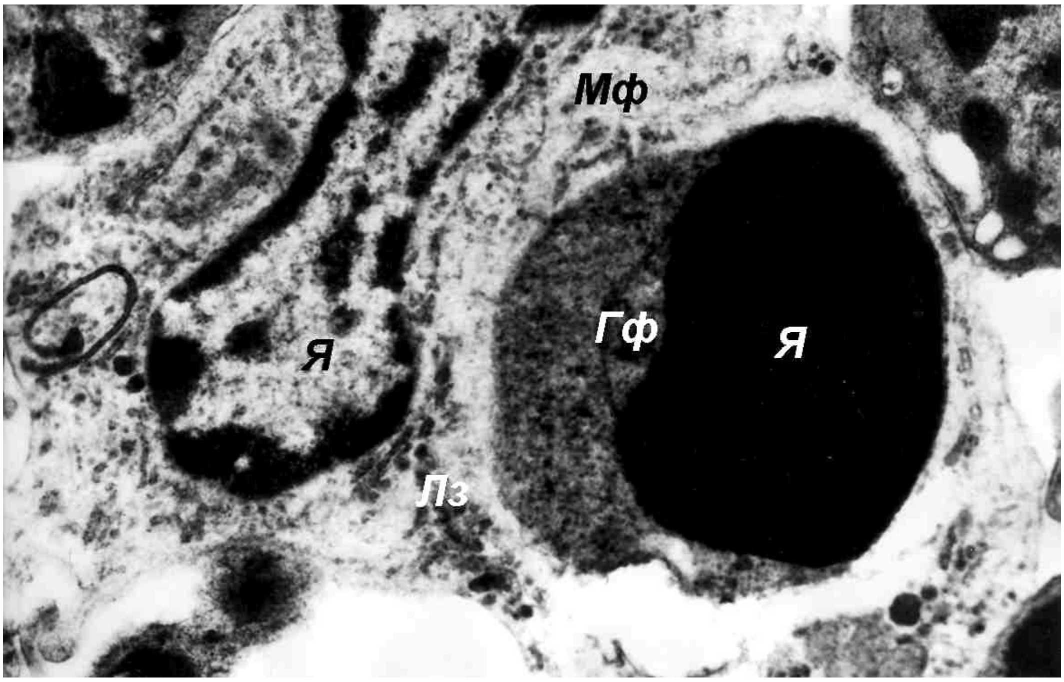


Fig. 3.24. Thymus of a rat with long-term hypothyroidism during the prepubertal period. A macrophage with a phagocytized thymocyte. TEM, magnification x20000.

These findings suggest that in rats with prolonged hypothyroidism, an active process of thymocyte degradation occurs, accompanied by an increase in macrophage functional activity.

Conclusion of Chapter 3

The analysis of thyroid hormones (T4, T3) and thyroid-stimulating hormone (TSH) levels in prepubertal rats subjected to short-term and long-term experimental hypothyroidism revealed significant alterations in hormone concentrations. Even in cases of short-term hypothyroidism, serum triiodothyronine (T3) levels were reduced by more than half, while thyroxine (T4) concentrations dropped by nearly 1.6 times. In contrast, thyroid-stimulating hormone (TSH) levels in this group of rats nearly doubled compared to the control group. This increase in TSH reflects the classical feedback mechanism, where a decline in thyroid hormone levels triggers the release of pituitary tropic hormones to compensate.

The mercazolil-induced hypothyroidism model demonstrated a marked suppression of thyroid hormone synthesis and secretion, despite the elevated TSH levels. While short-term hypothyroidism caused a moderate decrease in thyroid hormone concentrations, long-term hypothyroidism led to more pronounced thyroid hypofunction and a more significant decline in hormone levels.

Regarding body and thymus weight, short-term hypothyroidism did not result in notable changes. However, in rats experiencing prolonged hypothyroidism during the prepubertal period, thymus weight was reduced by 38%. Despite this decline, no significant differences were observed in overall body weight between the control and experimental groups. Consequently, the thymus weight index—expressed as the ratio of thymus weight to body weight—was 40% lower in rats with long-term hypothyroidism than in the control animals. These findings indicate that extended thyroid dysfunction leads to thymic hypoplasia, reducing its mass while the overall body weight remains unchanged or slightly increases.

Morphometric analysis of the thymus revealed a reduction in the number of thymocytes in rats with short-term hypothyroidism, which was decreased by 20%. In rats with long-term hypothyroidism, there was a more pronounced reduction, with a 27% decrease in mature thymocytes compared to the control group. Both the cortical and medullary zones of the thymus were significantly smaller in rats with long-term hypothyroidism. The cortical zone was reduced by 39%, while the medullary zone shrank by 26%. Additionally, a shift of the cortical zone toward the periphery of the thymus was observed.

The results of the study showed that the thymus in hypothyroidism underwent characteristic morphofunctional changes in rats. These changes were manifested as thymocyte depletion and a significant increase in the number of macrophages and plasma cells in the cortical and medullary zones. A thorough study of the ultrastructure of the thymus in rats with hypothyroidism revealed a significant number of vacuoles in the cytoplasm of thymocytes. Additionally, macrophages and their transitional forms, characterized by large numbers of primary and secondary lysosomes, were often found.

Chapter VI. THYROID HORMONE INDICATORS, MORPHOMETRIC AND ULTRASTRUCTURAL FEATURES OF THE THYMUS IN EXPERIMENTAL HYPERTHYROIDISM

§4.1. Short-term and Long-term Hyperthyroidism

The levels of thyroid and thyroid-stimulating hormones in the serum of rats during the prepubertal period under conditions of short-term and long-term hyperthyroidism are presented in Table 4.1.

Table 4.1 Concentration of T3, T4, and TSH in the serum of rats during experimental hyperthyroidism, induced in the prepubertal period (M ± m)

Animal Groups	**Hormones T3 (nmol/l)**	**T4 (mol/l)**	**TSH (mME/ml)**
Control Group (n=10)	2.4 ± 0.3	45.1 ± 4.0	0.2 ± 0.01
Short-term Hyperthyroidism (n=10)	4.2 ± 0.2 ***	76.3 ± 5.2 ***	0.1 ± 0.01 ***
Long-term Hyperthyroidism (n=10)	6.5 ± 0.4 ***^^^	98.7 ± 6.4 ***^^	0.08 ± 0.01 ***

Note: * - differences from the control group are significant (*** - P<0.001); ^ - differences from the short-term hyperthyroid group are significant (^^ - P<0.01, ^^^ - P<0.001)

The table indicates that short-term hyperthyroidism resulted in a more than 1.7-fold increase in the serum concentration of triiodothyronine (T3), while thyroxine (T4) levels rose by nearly 1.6 times when compared to the control group. Conversely, the thyroid-stimulating hormone (TSH) concentration decreased by almost 2 times in this group, reflecting the classic feedback mechanism. This mechanism dictates that elevated levels of exogenous thyroid hormones suppress the secretion of their corresponding tropic hormone (in this case, TSH) from the pituitary gland.

Further analysis reveals that long-term hyperthyroidism led to a more substantial increase in thyroid hormones, with T3 levels increasing by a factor of 2.7, and T4 levels rising by 2.2 times relative to the control group. During long-term hyperthyroidism, the concentration of TSH dropped by 2.5 times compared to baseline levels.

The experimental model of hyperthyroidism induced by L-thyroxine resulted in a significant rise in thyroid hormone levels in the bloodstream, demonstrating that the administration of external thyroid hormones effectively inhibits TSH secretion from the pituitary gland. In cases of short-term hyperthyroidism, TSH levels were reduced by nearly half, whereas prolonged hyperthyroidism led to more substantial endocrine changes, including an over threefold increase in thyroid hormone levels and a 2.5-fold decline in TSH levels compared to the control group.

Furthermore, the study analyzed thymus mass in animals exposed to hyperthyroidism during the prepubertal period. The relevant findings are summarized in **Table 4.2**.

Table 4.2 Thymus mass in experimental animals subjected to hyperthyroidism during the prepubertal period (M ± m).

Animal Groups	**Animal Body Mass (g)**	**Thymus Mass (mg)**	**Thymus Index (mg/g)**
Control (n=17)	154.7 ± 7.8	132.2 ± 5.3	0.85 ± 0.03
Short-term Hyperthyroidism (n=16)	150.5 ± 8.4	138.4 ± 6.3	0.92 ± 0.04
Long-term	152.7 ± 7.4	148.5 ± 4.7 *	0.97 ± 0.03 *

Hyperthyroidism (n=16)			

Note: * - Significant differences compared to the control group (* - $P<0.05$).

Thus, the L-thyroxine-induced hyperthyroidism model demonstrated a notable increase in serum thyroid hormones, while the concentration of pituitary TSH progressively declined. Short-term hyperthyroidism was characterized by a moderate elevation in thyroid hormone levels, whereas long-term hyperthyroidism resulted in a more pronounced hormonal imbalance.

Morphometric analysis revealed that the average area of thymic lobules and its various zones significantly changed during experimental hyperthyroidism induced during the prepubertal period (Table 4.3).

Table 4.3 Average area of lobules, cortical, and medullary zones of the thymus during experimental hyperthyroidism induced in the prepubertal period ($M \pm m$, x10^5 μm²)

Area	Total Lobule Area	Cortical Zone	Medullary Zone
Control (n=17)	21.5 ± 0.6	15.9 ± 0.2 (73%)	5.6 ± 0.1 (26%)
Short-term Hyperthyroidism (n=16)	21.7 ± 0.4	16.4 ± 0.3 (75%)	5.3 ± 0.2 (24%)
Long-term Hyperthyroidism (n=16)	23.6 ± 0.3*	18.5 ± 0.2* (78%)	5.1 ± 0.1* (21%)

Note: * - differences from the control group are significant (* - $P<0.05$)

The data showed that the total thymic area remained practically unchanged during short-term hyperthyroidism, while during long-term hyperthyroidism, the thymus area increased by almost 10% compared to the control. The enlargement of the thymus area was predominantly driven by the expansion of the cortical zone, which increased by 11%, while the medullary zone exhibited a slight tendency to contract. The noticeable expansion of the cortical region, alongside a minor reduction in the medullary zone, suggests that prolonged hyperthyroidism induces mild thymic hyperplasia, with the most pronounced changes occurring in the cortical zone.

Additionally, we determined the average cellular density within the thymic lobules per unit area (Table 4.4).

Table 4.4
Average cell density in thymic lobules under experimentally induced hyperthyroidism during the prepubertal period (M ± m, $\times 10^2$ cells per 10^5 μm²).

Zone	Cortical Zone	Medullary Zone
Control (n=17)	19.1 ± 0.28	9.7 ± 0.17
Short-term Hyperthyroidism (n=16)	20.6 ± 0.22***	9.8 ± 0.31
Long-term Hyperthyroidism (n=16)	23.2 ± 0.18***^^^	11.3 ± 0.15***^^^

Note: * Indicates statistically significant differences from the control group (* - P<0.05); ^ Represents differences from the short-term hyperthyroid group that are not statistically significant (P>0.05).

The findings indicate that in rats subjected to long-term hyperthyroidism, the average cell density in the cortical zone increased by over 20% compared to control animals. Meanwhile, short-term hyperthyroidism resulted in a comparatively smaller increase of about 9%. A notable rise in cell density within the medullary zone was observed only in long-term hyperthyroidism, showing a 15% increase relative to the control group.

A detailed analysis of the thymic cytogram demonstrated that short-term hyperthyroidism significantly influenced the cellular composition of both the cortical and medullary zones (as shown in Table 4.5). A considerable increase in the number of lymphoblasts was recorded in both regions, accompanied by a notable rise in monocytes, macrophages, and tissue granulocytes. The most notable increase in the number of monocyte-macrophage cells occurred in the cortical zone, whereas the medullary zone saw a rise in plasma cells, tissue basophils, and granulocytes. Additionally, a significant increase in the number of epithelial-reticular cells (ERCs) was noted, which contrasts with the typical observations under hypothyroid conditions.

In the cortical zone, which occupies the majority of the thymic lobes, the presence of large Golgi bodies was also notable, as seen in Figure 4.2. These cellular changes suggest a complex adjustment in thymic cell populations in response to hyperthyroidism, which may have implications for thymic function and overall immune response in these animals.

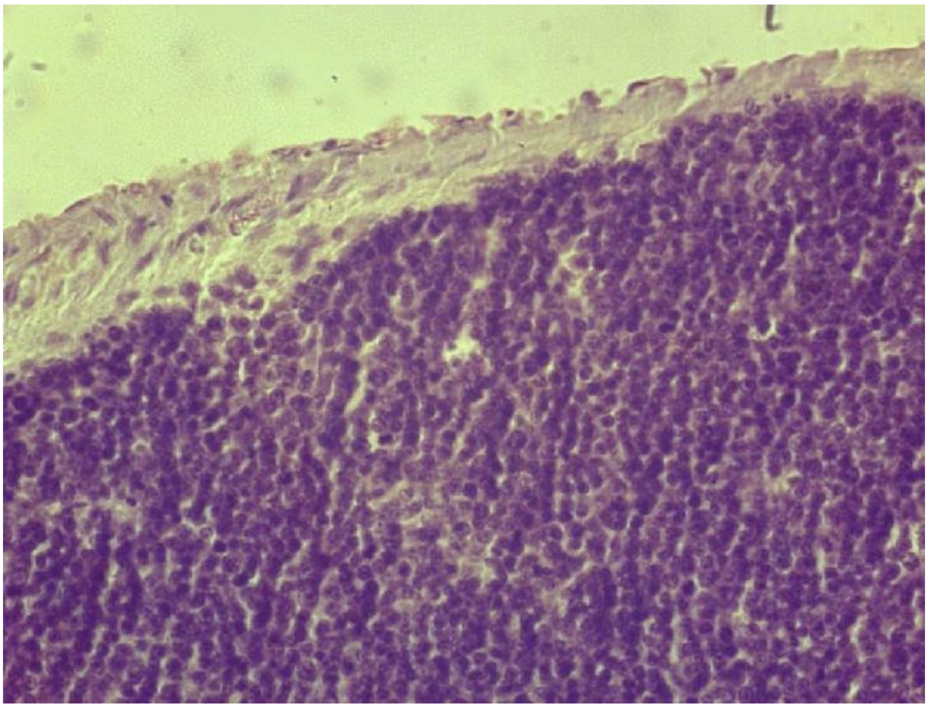


Figure 4.1. Thymus of a rat with short-term hyperthyroidism during the prepubertal period. Capsule and cortical zone. Approx. magnification: 20x, 10x.

Ultrastructural studies also showed no significant submicroscopic changes in the thymus under conditions of short-term hyperthyroidism induced in the prepubertal period. The epithelial-reticular cells (ERC) of the thymus were characterized by large size and numerous cytoplasmic extensions. Both in the body of the ERC and in its cytoplasmic processes, besides the usual organelles, secretory vacuoles and tonofibrils were identified (Figure 4.3). The secretory vacuoles varied widely in size, some containing moderate amounts of electron-dense, fine-granular material. Thymocytes closely adhered to both the body and the cytoplasmic processes of the ERC. Among the thymocytes, cells in a state of mitotic division were frequently observed (Figure 4.4). In the cortical zone, macrophages containing large lysosomes in their cytoplasm were detected. The blood vessels of the thymus exhibited heterogeneous structures.

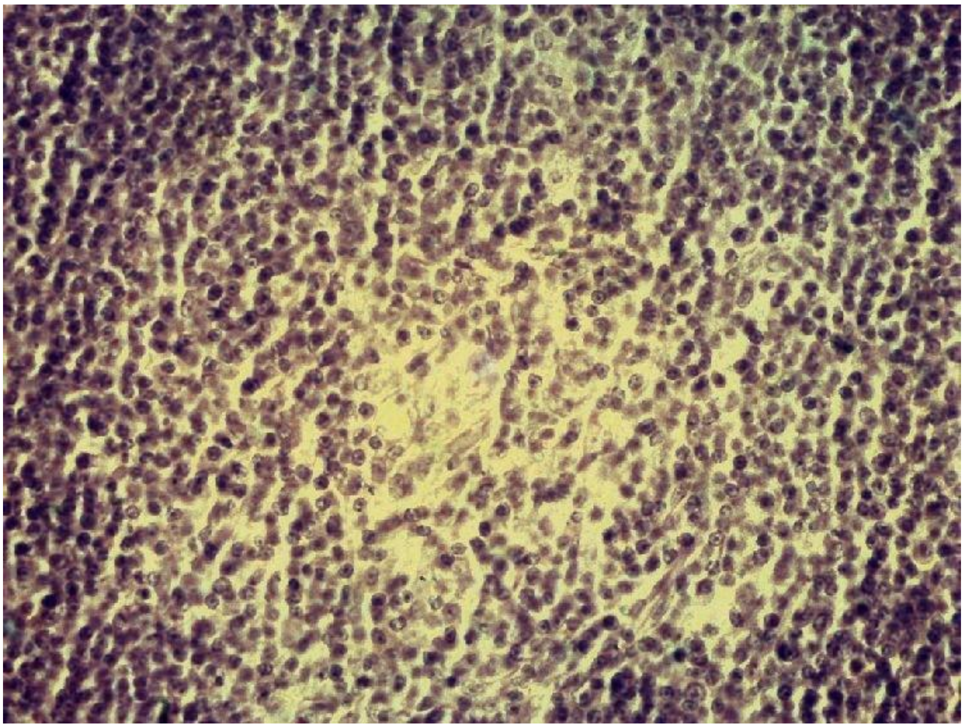
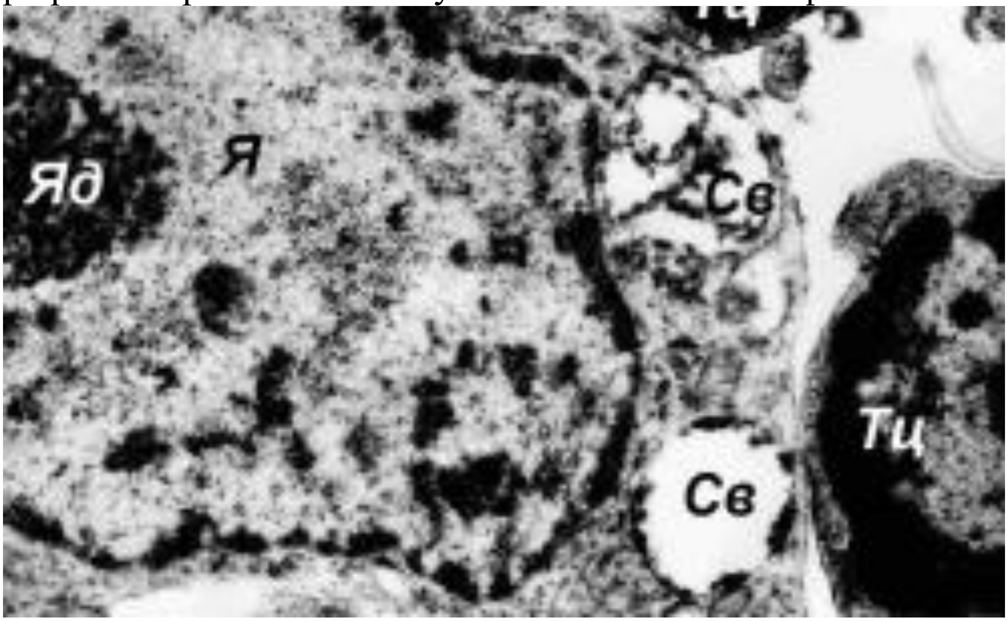


Figure 4.2. Thymus of a rat with short-term hyperthyroidism during the prepubertal period. Medullary zone with Hassall's corpuscle in the center.



Approx. magnification: 20x, 10x.

Figure 4.3. Thymus of a rat with short-term hyperthyroidism during the prepubertal period. ERC of the cortical zone of the thymus. TEM, x24000.

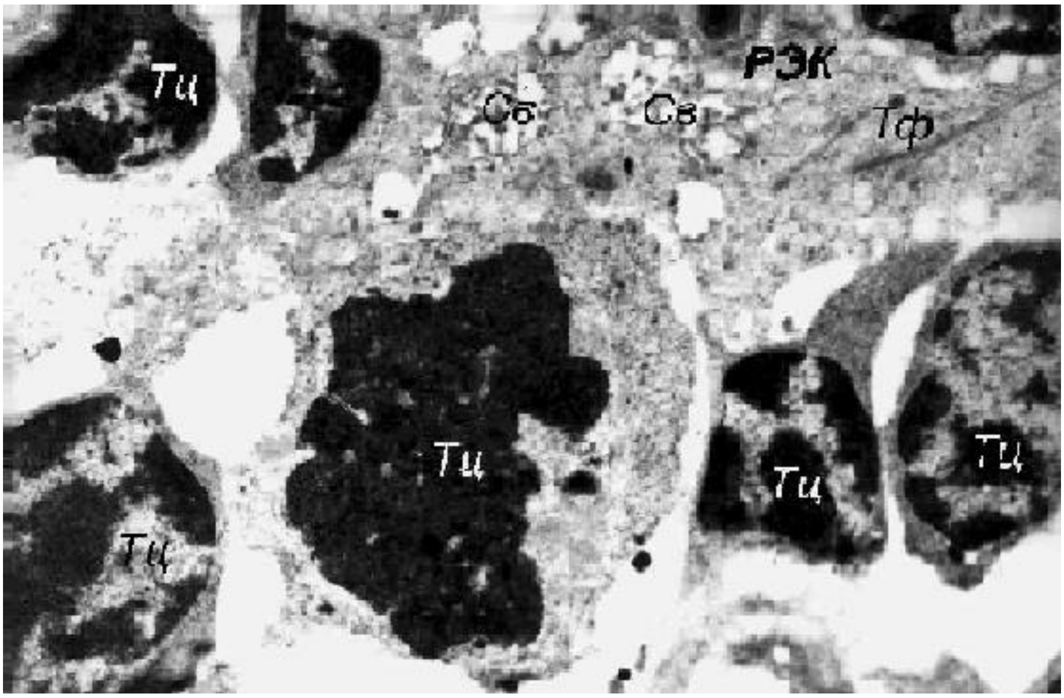


Figure 4.4. Thymus of a rat with short-term hyperthyroidism during the prepubertal period. ERC surrounded by thymocytes. TEM, x22000.

Given that this group of animals occasionally exhibited a few destructive thymocytes, mostly located in the cortical zone of the thymus, the structural analysis revealed that in experimental short-term hyperthyroidism, no significant morphological or ultrastructural changes were observed in the thymus. This was consistent with the morphometric data described above for this group of animals.

The morphological changes in the thymus were somewhat more pronounced in rats with long-term experimental hyperthyroidism. Isolated areas of edema and infiltration by mononuclear cells in the capsule and interlobular connective tissue were often observed (Figure 4.5). In most lobules, the cortical zone was thickened and formed a broad strip. Thymocytes in the cortical zone were tightly packed, with distinct ERCs and macrophages containing inclusions in their cytoplasm (Figure 4.6). Only in a few lobules in the cortical zone were light areas containing destructive thymocytes found. Frequently, macrophages with dense inclusions in their cytoplasm were observed in the cortical and sometimes the medullary zones. Localized dilation of blood vessels and their congestion were noted, especially in the cortico-medullary zone, which was rich in vessels (Figure 4.7). The medullary zone predominantly appeared light due to the low density of thymocyte distribution. Hassall’s corpuscles of varying sizes and structures were frequently observed within it (Figure 4.8).

Thus, long-term hyperthyroidism in prepubertal rats was associated with certain structural changes in the thymus, which manifested as moderate disorganization of the structural-functional zones of the lobules. The morphological changes were more pronounced in long-term hyperthyroidism, whereas no significant alterations were observed during short-term hyperthyroidism.

Electron microscopy revealed a certain dynamic of submicroscopic changes in thymocytes and thymic microenvironment cells during long-term hyperthyroidism. More significant ultrastructural changes were also detected in rats with long-term hyperthyroidism. This group showed marked destruction of thymocytes, particularly in the cortical zone, and high functional activity of the monocyte-macrophage system. Thymocytes resembling prolymphocytes and large lymphocytes with vacuolated cytoplasm were frequently found (Figure 4.9). The epithelial-reticular cells of the cortical zone mostly displayed normal ultrastructure. However, the presence of large secretory vacuoles, either devoid of dense material or containing only a minimal amount, was observed (Figure 4.10). Thymocytes in direct contact with the plasma membrane of the ERC generally exhibited a normal ultrastructure characteristic of medium and small lymphocytes.

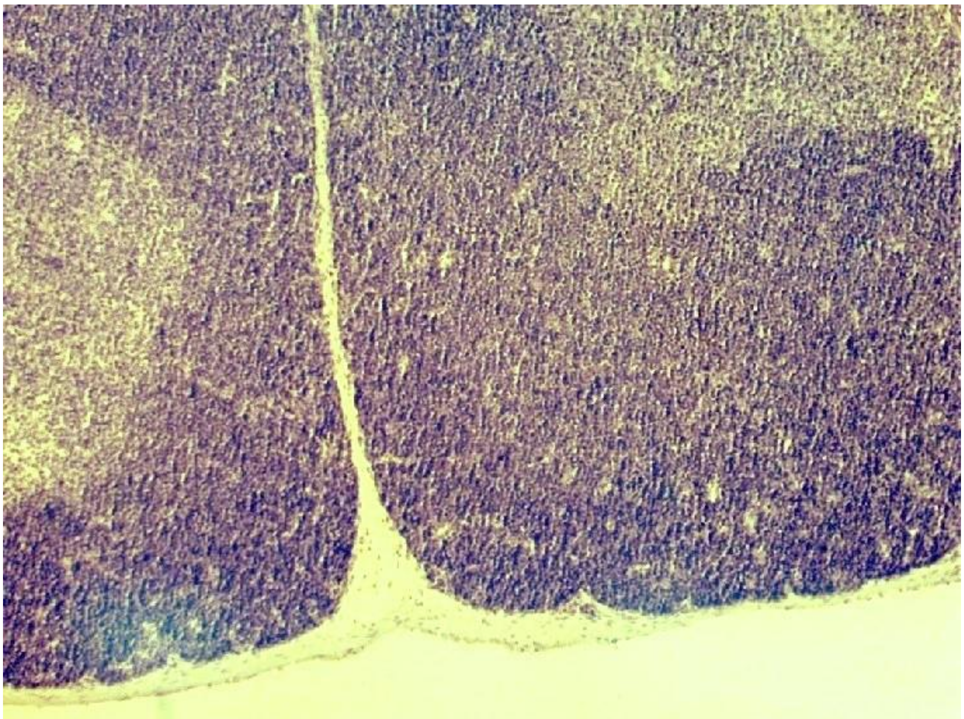


Figure 4.5. Thymus of a rat experiencing extended hyperthyroidism during the prepubertal stage, highlighting interlobular connective

tissue and the cortical region. Approximate magnification: 20x, 10x.

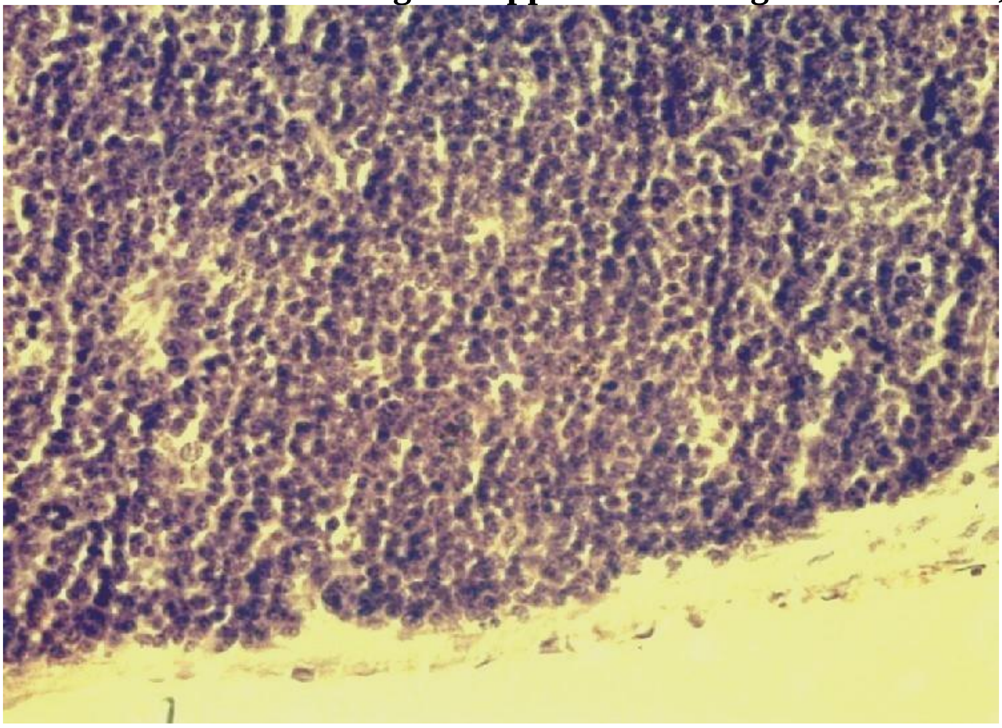


Figure 4.6. Thymus of a rat subjected to prolonged hyperthyroidism during the prepubertal stage, displaying cortical hyperplasia with densely packed thymocytes, ERCs, and macrophages. Approximate magnification: 20x, 10x.

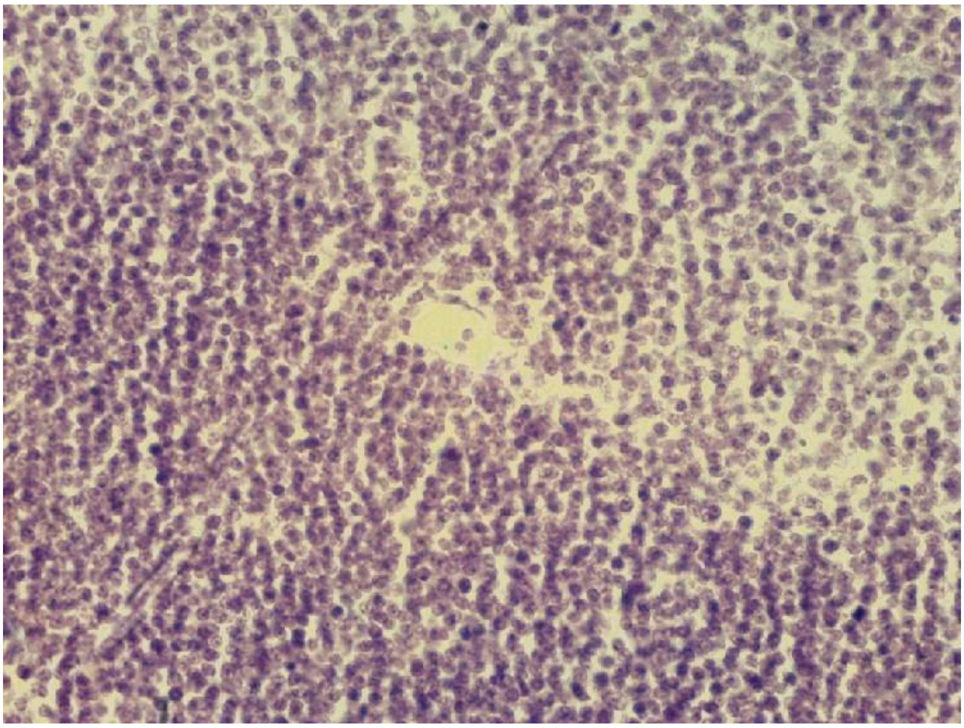


Figure 4.7. Thymus of a rat exposed to prolonged hyperthyroidism during the prepubertal period, showing the cortico-medullary zone with dilated blood vessels. Approximate magnification: 20x, 10x.

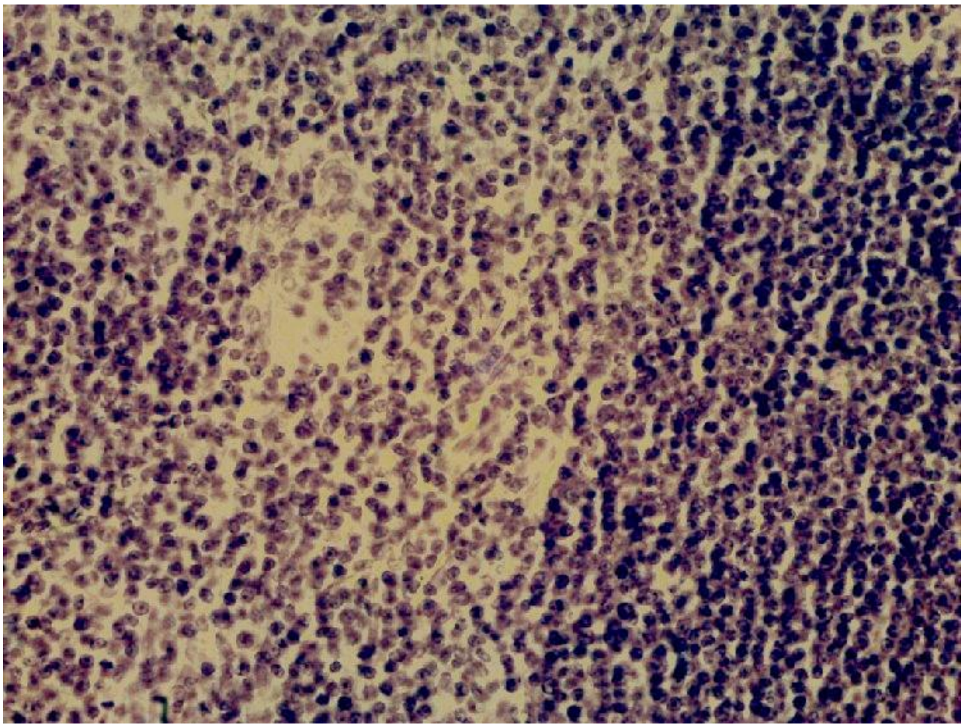


Figure 4.8. Thymus of a rat subjected to prolonged hyperthyroidism during the prepubertal period, displaying the medullary zone containing Hassall's corpuscles and macrophages. Approximate magnification: 20x, 10x.

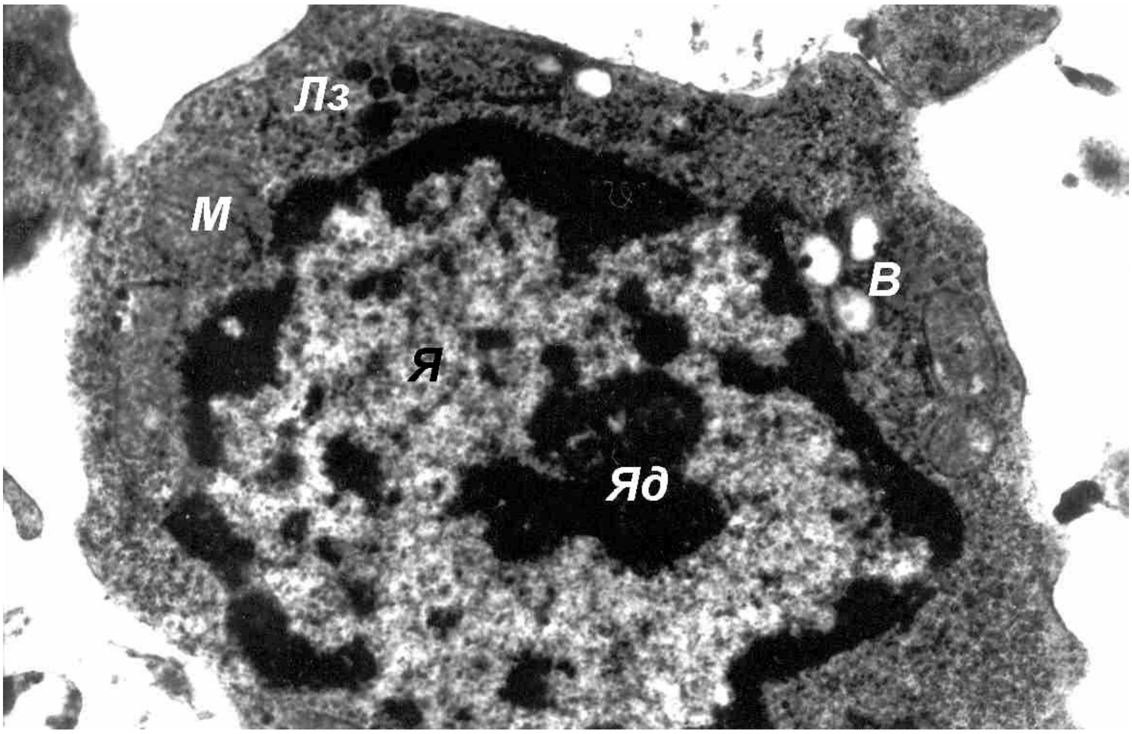


Figure 4.9. Thymus of a rat with long-term hyperthyroidism during the prepubertal period. Large thymocyte in the cortical zone with moderate vacuolization of the cytoplasm. TEM, x14000.

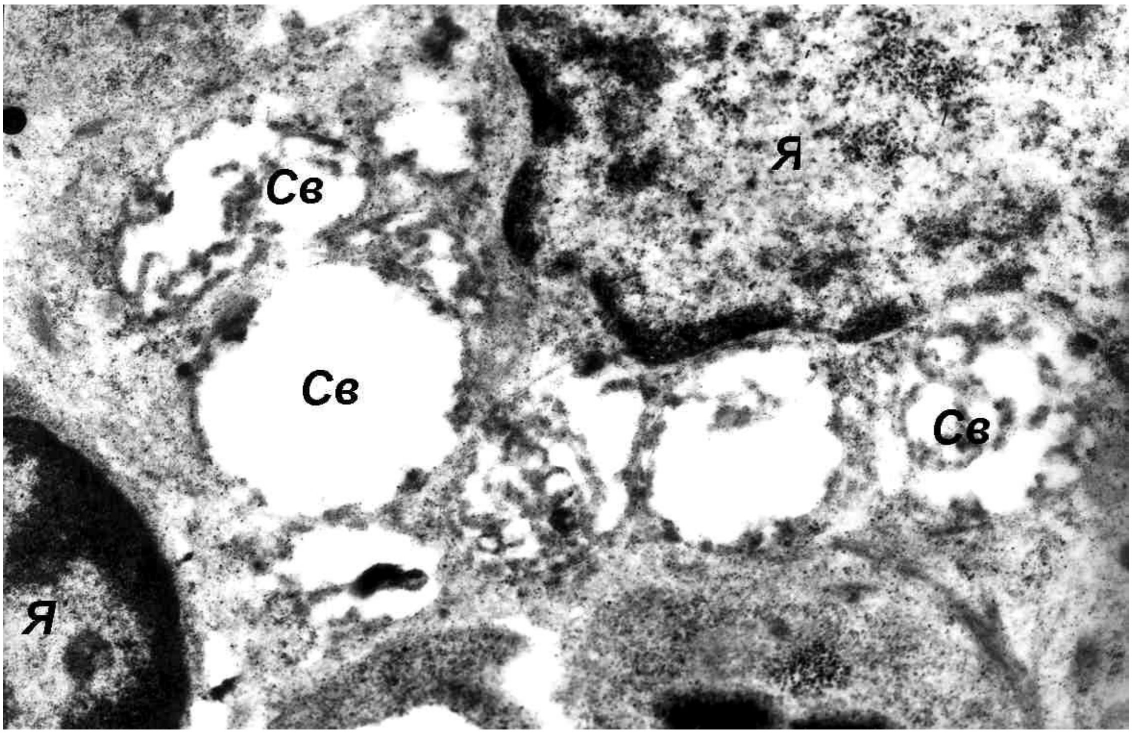


Figure 4.10. Thymus of a rat with long-term hyperthyroidism during the prepubertal period. ERC of the cortical zone. TEM, magnification x22000.

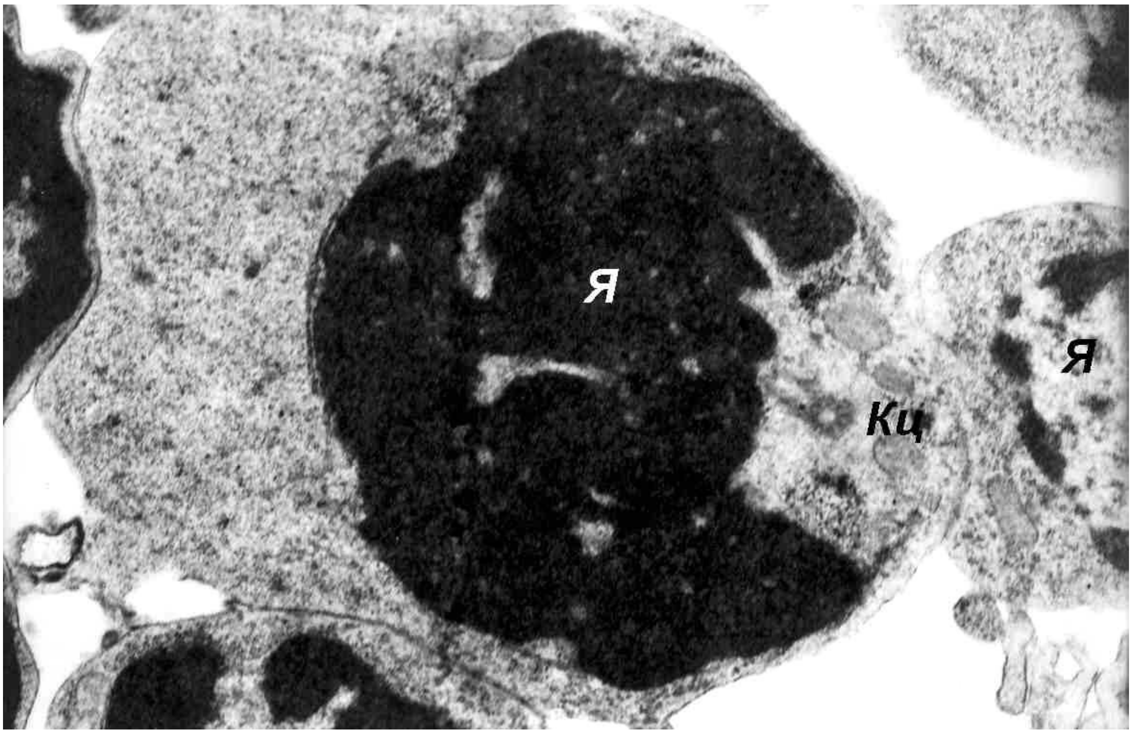


Figure 4.11. Thymus of a rat with long-term hyperthyroidism during the prepubertal period. Thymocyte undergoing mitotic division. TEM, magnification x14000.

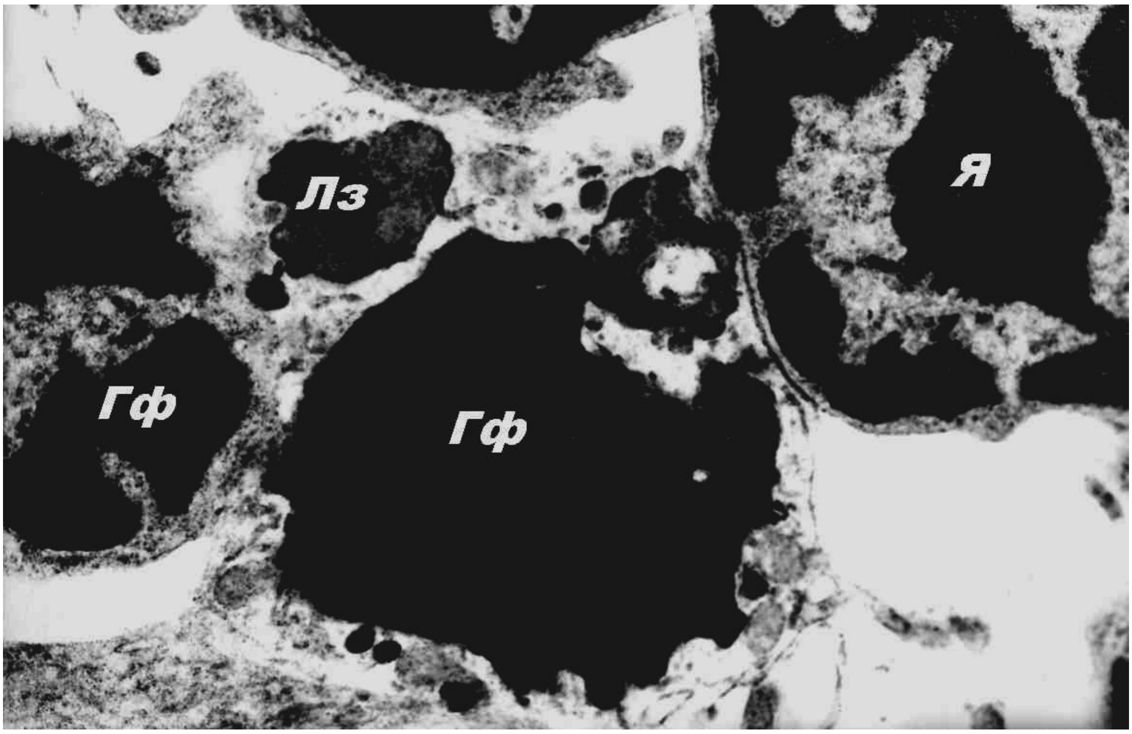


Figure 4.12. Thymus of a rat with long-term hyperthyroidism during the prepubertal period. Fragment of a macrophage's cytoplasm. TEM, magnification x22000.

Additionally, destructive changes in thymocytes, such as nuclear pyknosis and focal cytoplasmic lysis, were frequently observed. Thymocytes at various stages of mitotic division were also commonly seen (Figure 4.11). These cells exhibited a well-defined cell center with centrioles displaying typical ultrastructure. Macrophages in the cortical zone were large and contained both primary and secondary lysosomes of varying sizes and densities in their cytoplasm. Large heterophagosomes containing remnants of destructive thymocytes were also frequently observed in the cytoplasm of macrophages (Figure 4.12).

Conclusion of Chapter IV:

The experimental model of hyperthyroidism induced by L-thyroxine resulted in a significant elevation of thyroid hormone levels in the serum, accompanied by a progressive decline in thyroid-stimulating hormone (TSH) concentration. Short-term hyperthyroidism was associated with a moderate increase in thyroid hormone levels, whereas long-term exposure led to a more pronounced hyperthyroid state.

In the short-term hyperthyroid group, neither body weight nor thymus weight showed significant alterations, with only a slight and statistically insignificant increase in thymus mass and mass index. However, in cases of prolonged hyperthyroidism, moderate thymic hyperplasia was observed, accompanied by a slight increase in thymus mass, while overall body weight remained nearly unchanged compared to the control group.

Morphometric analysis indicated that the total average thymus area remained virtually unchanged in animals with short-term hyperthyroidism, whereas in those exposed to long-term hyperthyroidism, it increased by approximately 10% compared to the control group. This enlargement was primarily attributed to the cortical zone, which expanded by 11%, while the medullary zone exhibited a slight tendency to decrease. The proportion of brain area was measured at 24% in short-term hyperthyroid animals and 21% in those with long-term hyperthyroidism, in contrast to over 26% in the control rats.

Furthermore, histological analysis revealed that in rats subjected to long-term hyperthyroidism, the density of thymocytes in the cortical zone was more than 20% higher than in control animals. Meanwhile, during short-term hyperthyroidism, the increase in cell density was only about 9%. It was also noted that the number of epithelial-reticular cells (ERCs) was significantly increased, which had never been observed in hypothyroid conditions.

Short-term hyperthyroidism had little impact on the proliferation of cortical zone cells. In contrast, long-term hyperthyroidism significantly increased the number of mitotically dividing thymocytes in the cortical zone by 11%, with a small but unreliable tendency for an increase in the mitotic activity in the medullary zone. No statistically significant differences were found in the number of destructive thymocytes between the control and experimental animals.

Thus, hyperthyroidism during the prepubertal period was associated with thymus hyperplasia caused by increased proliferative activity of immature lymphoblasts and epithelial-reticular cells. These changes were more pronounced during long-term hyperthyroidism, as thyroid dysfunction continued until puberty.

In experimental short-term hyperthyroidism, no significant morphological or ultrastructural changes were observed in the thymus. In contrast, experimental long-term hyperthyroidism in prepubertal rats was accompanied by certain structural changes in the thymus, manifesting as moderate disorganization of the structural-functional zones of the lobules.

Therefore, in rats with long-term hyperthyroidism, thymus hyperplasia occurred overall, likely due to increased proliferative activity of undifferentiated precursor cells of thymocytes. At the same time, a tendency for increased thymocyte destruction was observed, which may be attributed to the excessive influence of thyroid hormones on the thymus.

Table 5.7
The number of mitotically dividing and destructive thymus cells in experimental hypo- and hyperthyroidism reproduced in sexually mature rats (M ± m, per 10^3 cells)

Cells	Mitotic		Destructive	
Animal Groups	Cortical zone | |	Medullary zone	Cortical zone |	Medullary zone |
Control (n=15)	47,4 ± 1,2	7,6 ± 0,8	15,3 ± 0,8	5,6 ± 0,4
Hypothyroidism (n=17)	34,5 ± 1,3***	6,8 ± 0,7	38,7 ± 2,2***	7,8 ± 0,3***
Hyperthyroidism (n=15)	56,2 ± 2,1***^^^	8,4 ± 0,5	17,2 ± 0,9^^^	6,8 ± 0,3*^

Note: * - Indicates statistically significant differences compared to the control group (*** - P < 0.001); ^ - Indicates statistically significant differences compared to the short-term hypothyroid group (^^^ - P < 0.001).

Light-optical and electron-microscopic studies of the thymus in experimental hypo- and hyperthyroidism revealed certain morphological rearrangements. These changes were largely identical to those observed in animals with prolonged hypo- and hyperthyroidism reproduced in the prepubertal period. However, the frequency of detection and the degree of morphological changes in sexually mature rats were significantly lower compared to groups of animals with hypo- and hyperthyroidism caused in the prepubertal period.

In experimental hypothyroidism in sexually mature rats, the histoarchitecture of the thymus was generally preserved. Often, swelling of the capsule and interlobular septa was observed, with infiltration by mononuclear cells. Blood vessels were dilated and blood-filled. In most lobules, the cortical zone appeared thinned, in the form of a narrow plate with loose arrangement of thymocytes (Fig. 5.1; Fig. 5.3). Large pale areas containing individual destructive thymocytes were frequently found in the cortical zone (Fig. 5.2). The medullary zone in the sections varied in size. In some lobules, Hassall’s corpuscles were identified.

Electron microscopy analysis revealed distinct ultrastructural changes in thymocytes and cells within the thymic microenvironment in sexually mature rats with hypothyroidism. These observations highlight various structural and functional changes at the cellular level due to thyroid hormone deficiency.

One of the primary findings involves the rough endoplasmic reticulum (RER) within the thymic cortex. In this region, the RER frequently contained large, dense formations of fine-grained material. This was indicative of disrupted cellular activity and dysfunction. Additionally, other secretory vacuoles within these cells exhibited a wide range of sizes, but most were nearly empty, devoid of any substantial internal material. This suggests an abnormality in the cell's ability to produce or maintain normal secretions.

Some thymocytes also displayed clear signs of cellular damage, including pyknosis (the condensation of the nucleus) and lysis (destruction) of the cytoplasm. These phenomena are classic indicators of cellular injury, which could result from the lack of proper thyroid hormones, affecting the normal thymic function.

The study also observed the presence of macrophages in the cortical zone, which were characterized by their varying sizes and the presence of numerous primary and secondary lysosomes of different sizes and densities within their cytoplasm. This suggests an active role of the macrophages in responding to tissue changes and damage. These macrophages were particularly prominent in areas where thymocytes were undergoing mass destruction. This pattern of damage indicates a highly active, though pathologically enhanced, immune response in the organ.

The destruction of thymocytes was sometimes so extensive that the affected cells were completely engulfed by macrophages. Once phagocytosed, the remnants of the thymocytes were contained within large heterophagosomes, structures within macrophages that held debris from the broken-down cells. These heterophagosomes revealed remnants of the destroyed thymocytes, emphasizing the macrophage’s role in the cellular cleanup process.

Interestingly, signs of thymocyte destruction were not limited to the cortical zone of the thymus. In some instances, such destruction was also observed within the medullary zone. This suggests that the effects of hypothyroidism may extend beyond just one region of the thymus, indicating a widespread influence on thymic structure and function.

The findings of this electron microscopy study underline the significant impact of experimental hypothyroidism on the thymus in sexually mature rats. It becomes clear that hypothyroidism leads to substantial structural-functional changes in the thymus. Most notably, the proliferative activity of cells in the cortical zone of the thymus is markedly reduced, which directly affects the growth and development of thymocytes. Simultaneously, the degree of thymocyte destruction in this area of the thymus increases.

Additionally, the activity of cells within the monocyte-macrophage system is significantly enhanced, leading to an increased level of phagocytosis targeting damaged thymocytes. These macrophages not only participate in clearing cellular debris but also contribute to the restructuring of thymic tissue. As a consequence, a reduction in the overall area of the thymic lobule is observed, accompanied by a decline in thymocyte density, particularly within the cortical zone. This decrease in thymocyte density likely reflects impaired thymic function and developmental capacity under hypothyroid conditions.

In conclusion, experimental hypothyroidism in sexually mature rats induces substantial cellular and structural modifications within the thymus. These changes include diminished thymocyte proliferation, increased cell destruction, heightened macrophage activity, and an overall reduction in thymic size and cell density. These findings not only enhance our understanding of the pathophysiology of hypothyroidism but also emphasize the essential role of thyroid hormones in preserving the integrity and functionality of the thymic microenvironment.

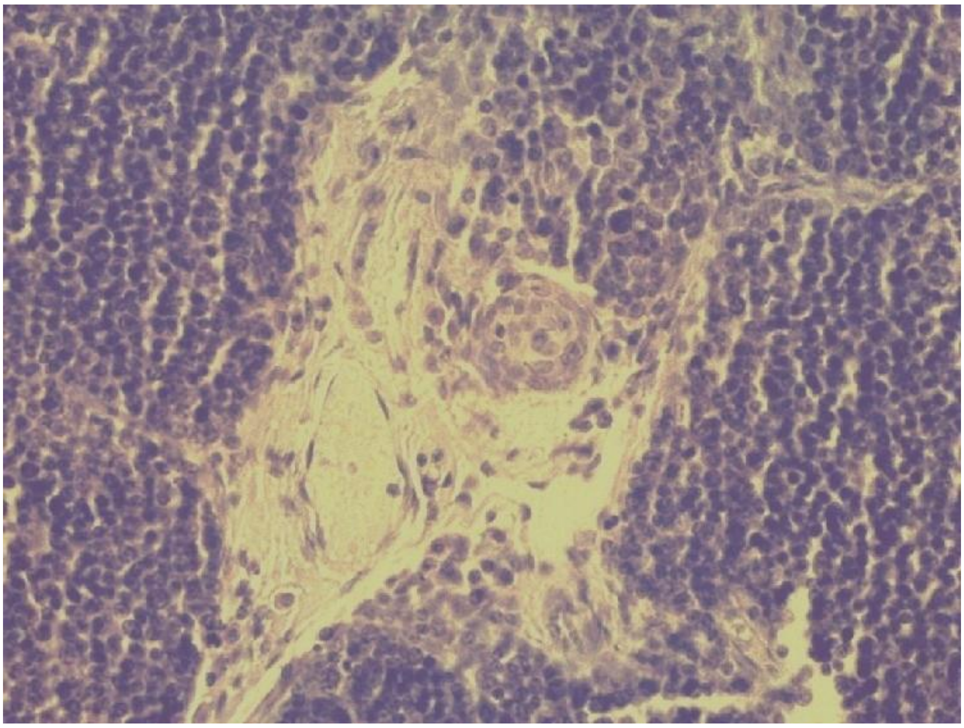


Fig. 5.1. Thymus of a sexually mature rat with hypothyroidism. Enlargement and blood engorgement of interlobular vessels, along with cortical zone thinning. Magnification: 20,000x, scale: 10 µm.

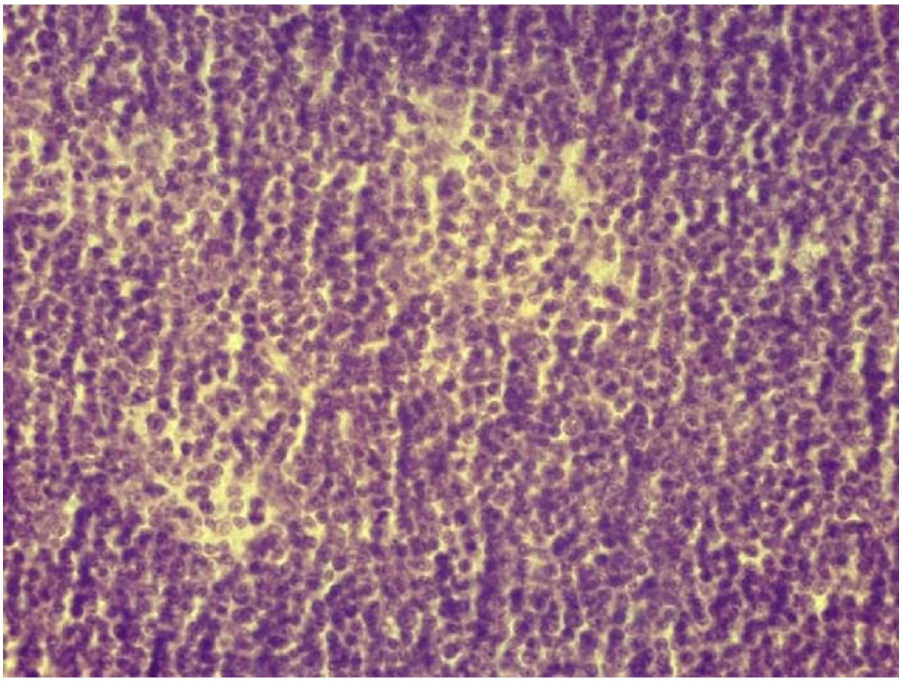


Fig. 5.2. Thymus of a sexually mature rat with hypothyroidism. Foci of destruction of thymocytes in the cortical zone. Magnification 20,000x, 10 µm.

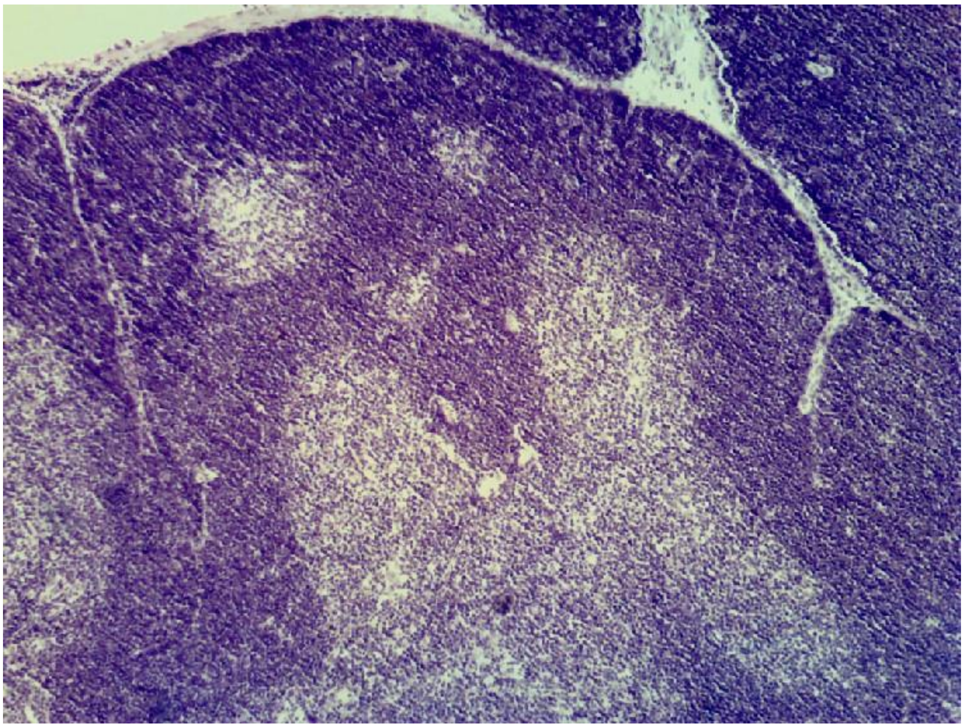


Fig. 5.3. Thymus of a sexually mature rat with hypothyroidism. Expansion, blood filling of blood vessels, reduced density of thymocytes in the cortical zone. Magnification 10,000x, 10 μm.

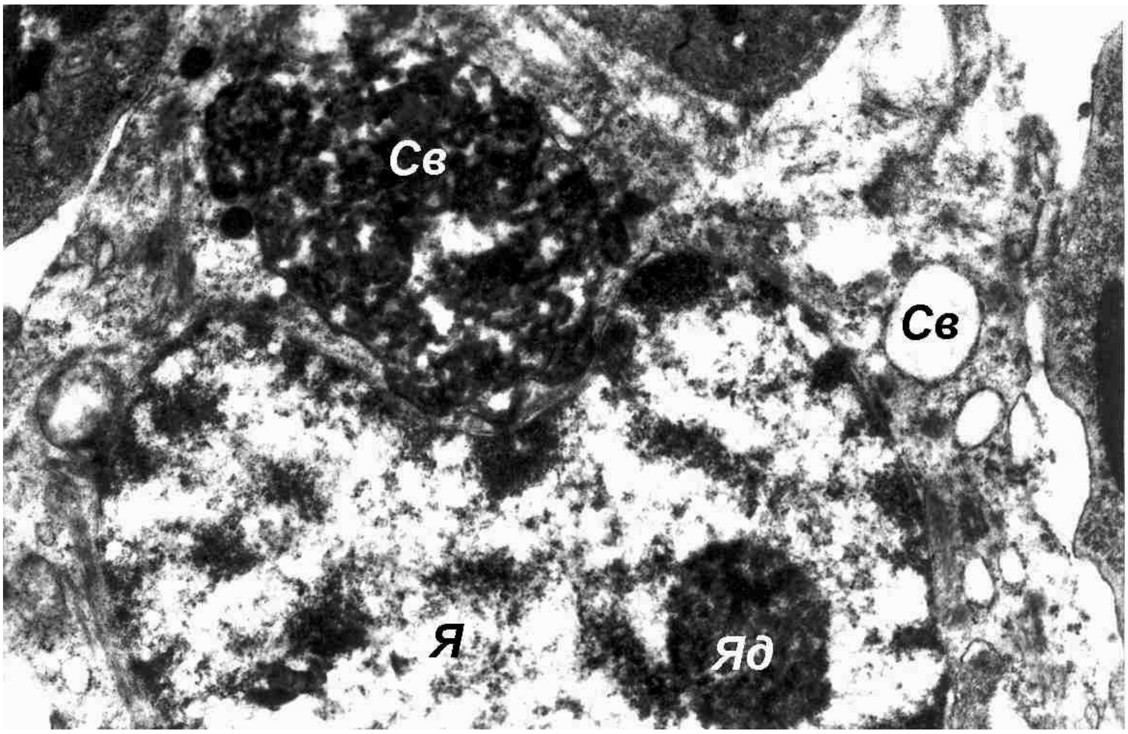


Fig. 5.4. Thymus of a sexually mature rat with hypothyroidism. RER of the cortical zone. In the cytoplasm, along with tonofibrils and small secretory vacuoles, a large dense formation is present. TEM, magnification x24,000.

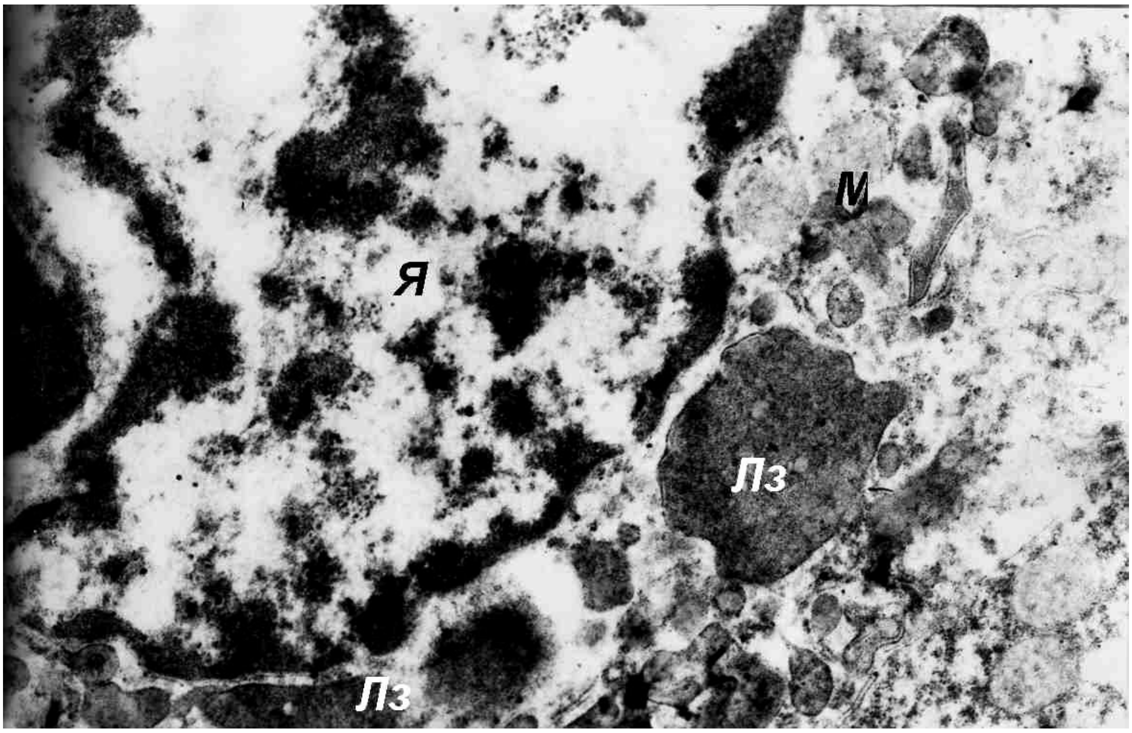


Fig. 5.5. Thymus of a sexually mature rat with hypothyroidism. Fragment of a macrophage in the cortical zone. TEM, magnification x24,000.

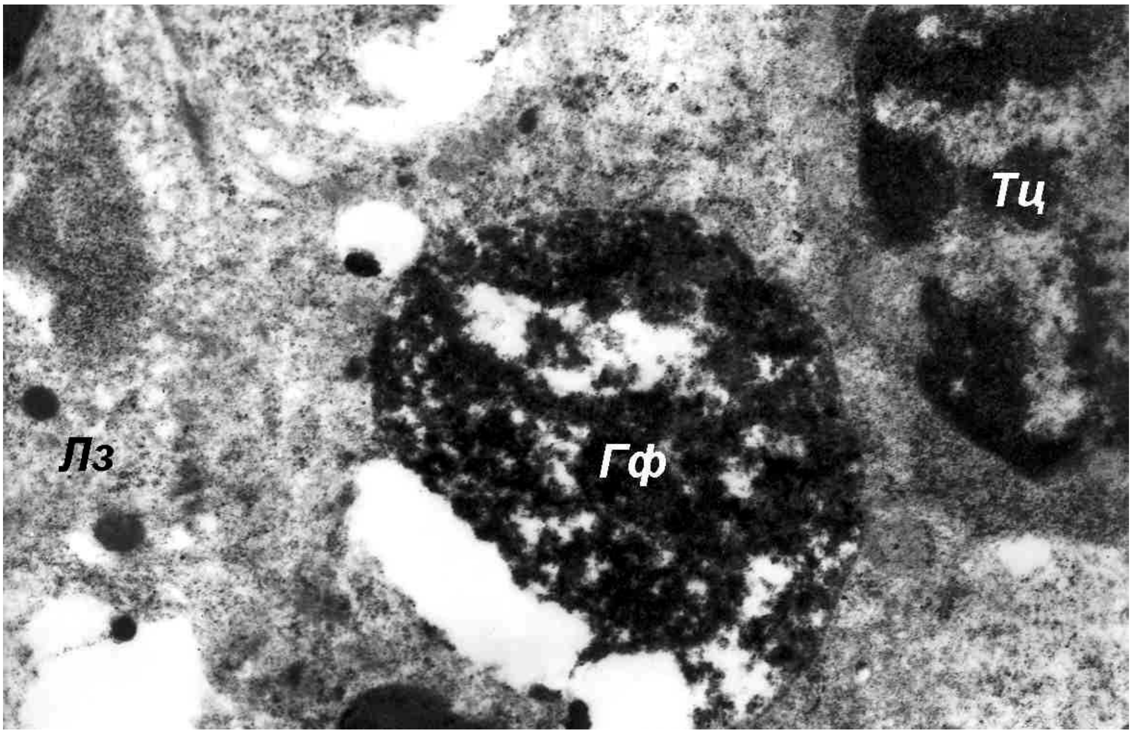


Fig. 5.6. Thymus of a sexually mature rat with hypothyroidism. Fragment of a macrophage in the cortical zone with a heterophagosome. TEM, magnification x24,000.

The obtained data indicate that not only prepubertal animals but also sexually mature individuals are sensitive to thyroid hormone deficiency.

Morphological and ultrastructural changes in the thymus in experimental hyperthyroidism in sexually mature animals are also largely identical to the changes described above in rats with prolonged hyperthyroidism during the prepubertal period.
Light-optically, complete preservation of the thymus histoarchitecture was observed, and in some cases, areas of edema, infiltration of the capsule and interlobular connective tissue with mononuclear cells were detected.
In most lobules, the cortical zone was thickened, with dense arrangement of thymocytes (Fig. 5.7). Among the thymocytes, light RERs and macrophages with inclusions in the cytoplasm were highlighted. Very rarely, foci of thymocyte lysis were detected in the cortical zone. Occasionally, blood vessels were dilated and filled, especially in the cortico-medullary zone, which is rich in vessels (Fig. 5.8). The medullary zone was mostly relatively small in size and appeared light due to the low density of thymocytes. Hassall's corpuscles, varying in size and structure, were relatively rarely found.

In experimental hyperthyroidism in sexually mature rats, moderate structural changes were noted in the thymus. These changes were confirmed through both light microscopy and morphometric analysis, which were further validated by electron microscopy. In this condition, there was a slight increase in the destruction of thymocytes, particularly in the cortical region, alongside heightened activity of the monocyte-macrophage system. The epithelial-reticular cells in the cortical zone largely retained their normal ultrastructure, though a few contained large secretory vacuoles filled with dense material.

Thymocytes in close proximity to the epithelial-reticular cells primarily exhibited the typical structure of medium and small lymphocytes. Occasionally, thymocytes displayed signs of damage, including pyknosis (nuclear condensation) and localized lysis of the cytoplasm. However, these destructive changes were infrequent. A notable observation was the presence of thymocytes undergoing mitotic division, which were frequently seen in various stages of the process. These mitotic cells displayed a well-formed centrosome and centrioles with a characteristic ultrastructure.

The capillaries in the cortical and cortico-medullary zones showed flattened endothelial cells and intact basal membranes, indicating that vascular integrity remained largely unaffected by the hyperthyroid condition.

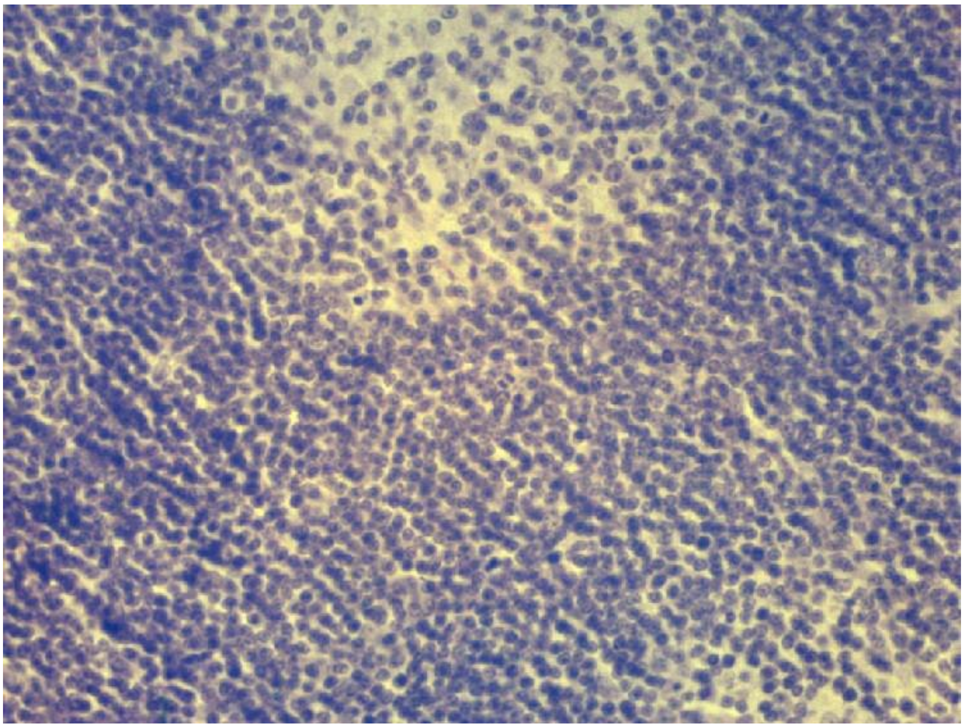


Fig. 5.7. Thymus of a sexually mature rat with hyperthyroidism. Cortical and medullary zones of the thymus. Magnification 40,000x, 10 μm.

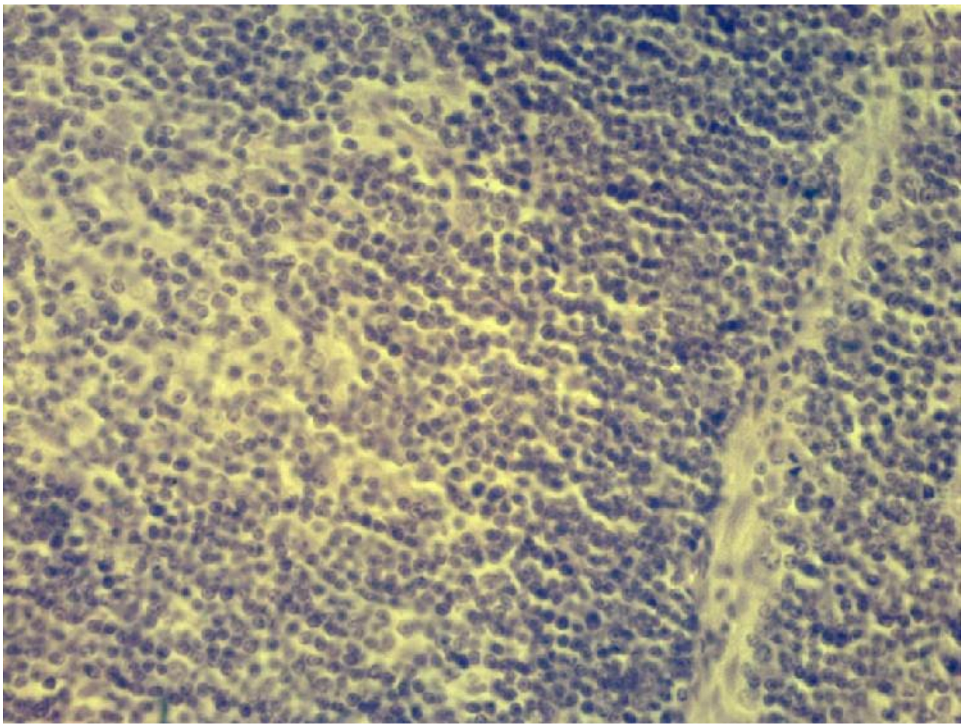


Fig. 5.8. Thymus of a sexually mature rat with hyperthyroidism. Cortico-medullary zone of the thymus. Magnification 20,000x, 10 μm.

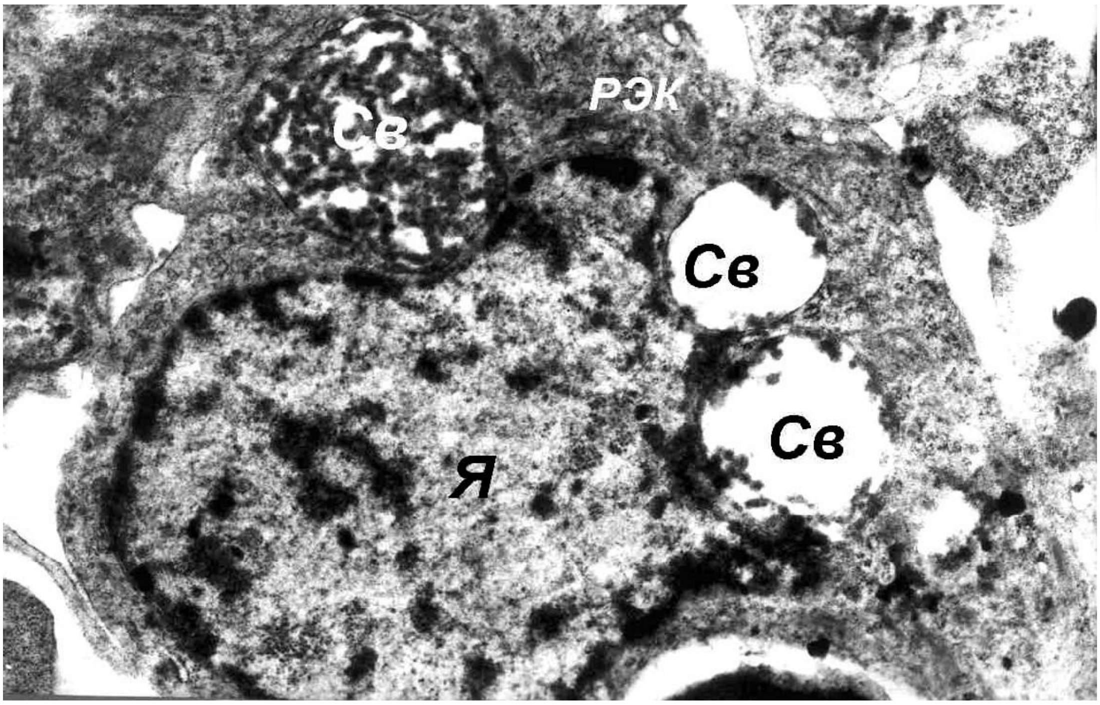


Fig. 5.9. Thymus of a sexually mature rat with hyperthyroidism. RER of the cortical zone. TEM, magnification x22,000.

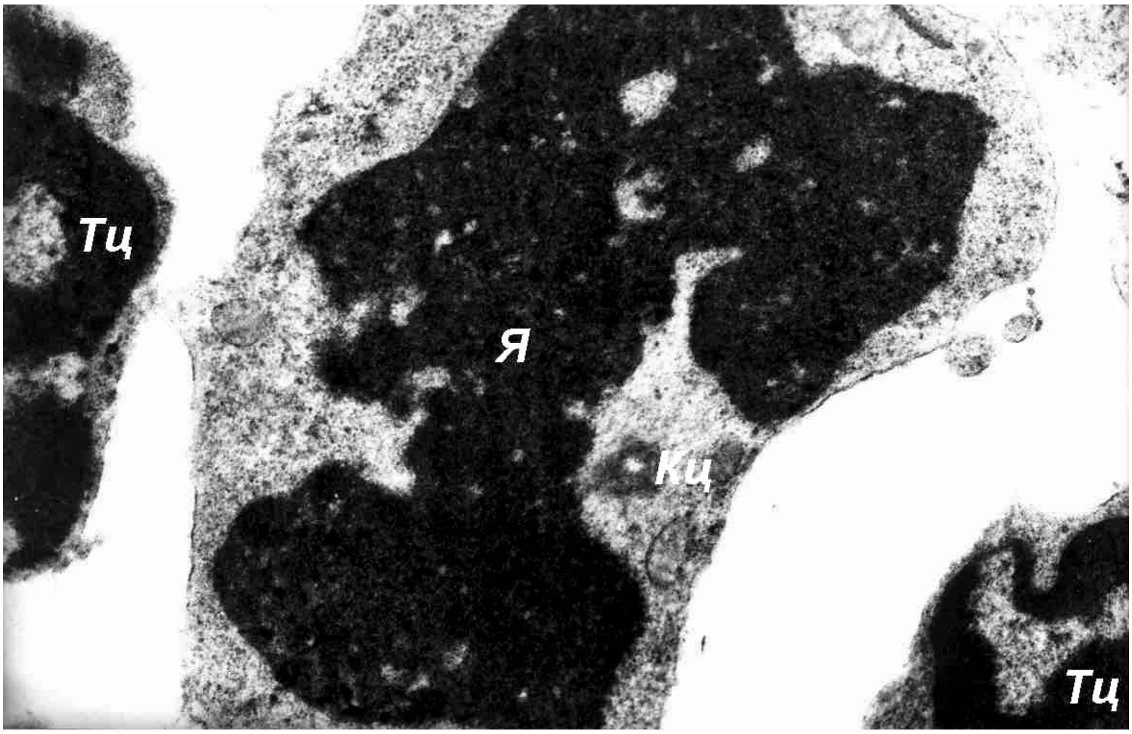


Fig. 5.10. Thymus of a sexually mature rat with hyperthyroidism. Thymocyte of the cortical zone in a state of mitotic division. TEM, magnification x18,000.

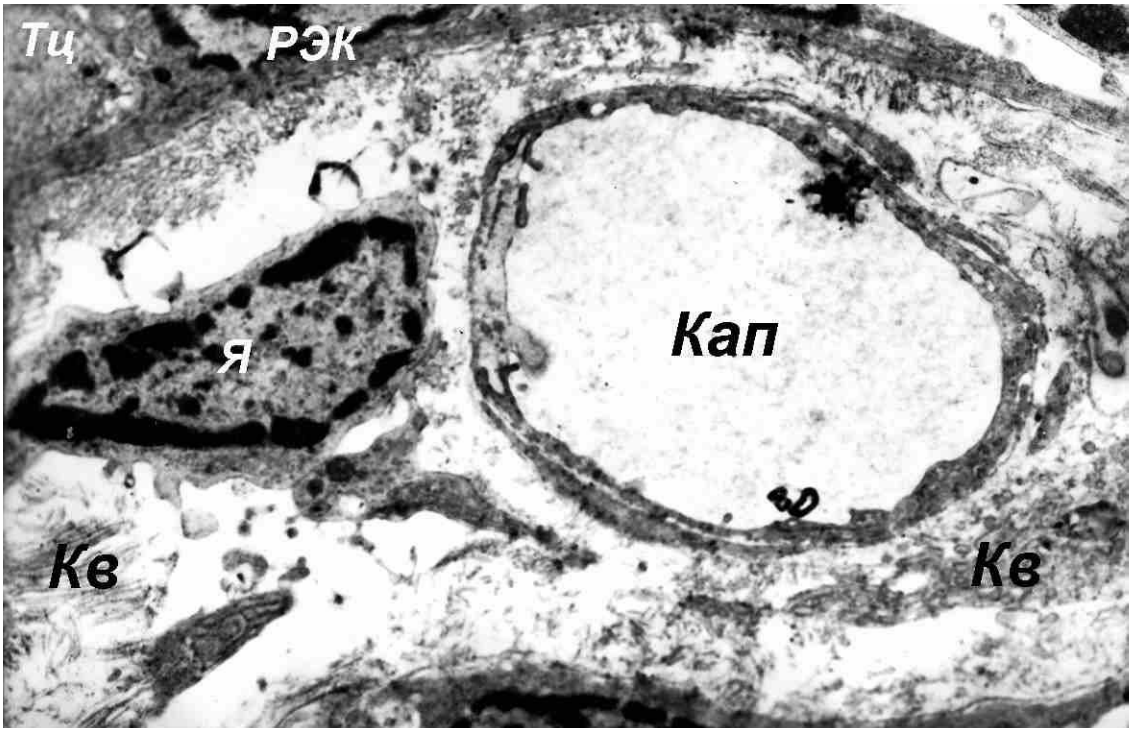


Fig. 5.11. Thymus of a sexually mature rat with hyperthyroidism. Blood capillary of the cortico-medullary zone of the thymus. TEM, magnification x12,000.

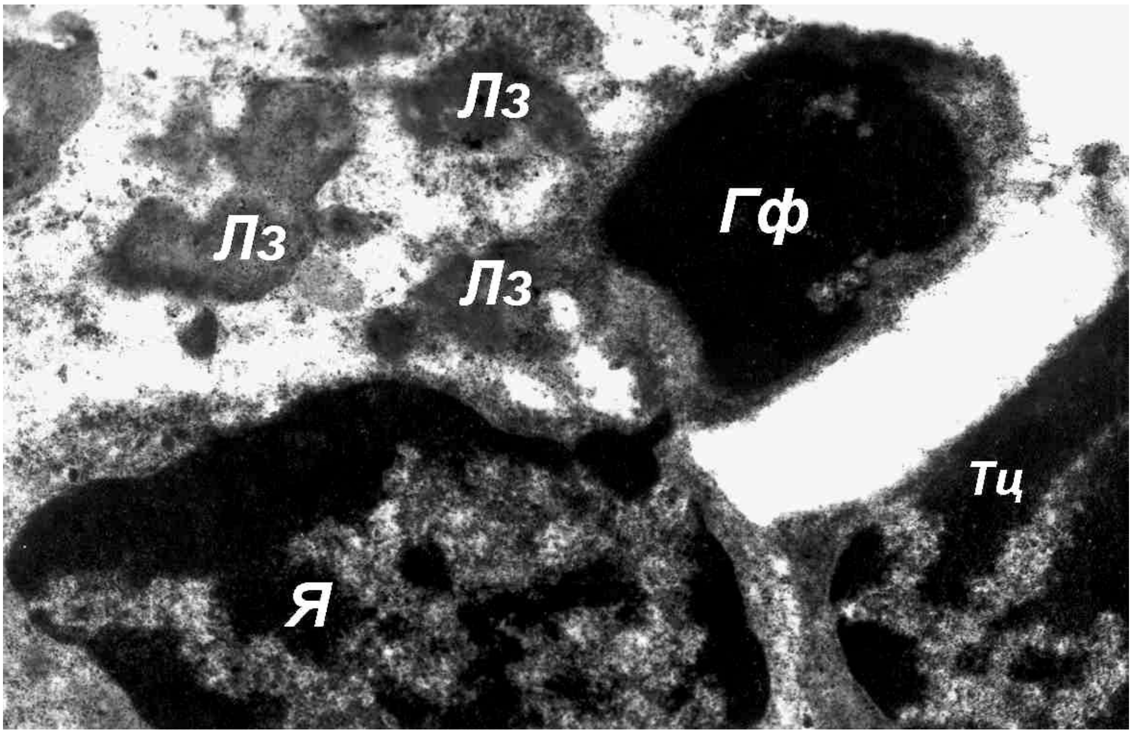


Fig. 5.12. Thymus of a sexually mature rat with hyperthyroidism. In the heterophagosome of a macrophage, remnants of a destructive thymocyte are identified. TEM, magnification x20,000.

Around the capillaries, components of the blood-thymic barrier – basal membranes, connective tissue, and processes of epithelial-reticular cells – were clearly defined (Fig. 5.11). Macrophages in the cortical zone were large, and their cytoplasm contained primary and secondary lysosomes of various sizes and densities. In the cytoplasm of macrophages, large heterophagosomes were often identified, in which the remnants of destroyed thymocytes were present (Fig. 5.12).

Experimental hyperthyroidism in sexually mature rats led to moderate alterations in the thymus. The changes that occurred were less pronounced than those seen in hypothyroidism. Thymocyte proliferation was somewhat reduced, and the degree of destruction of thymocytes in the cortical zone increased slightly. At the same time, the number of mitotic figures in the cortical zone was significantly higher than in hypothyroidism. The macrophage system in the cortical zone of the thymus was activated, leading to an increased process of phagocytosis of destructive thymocytes. The secretion activity of thymic epithelial-reticular cells increased.

Conclusion

Thus, experimental hypo- and hyperthyroidism in sexually mature rats led to a series of changes in the thymus, primarily a reduction in the number of mitotic thymocytes and an increase in the degree of thymocyte destruction. At the same time, changes in the macrophage system and the epithelioreticular cells of the thymus cortex were observed. In experimental hypothyroidism, the changes were more pronounced than in hyperthyroidism. These results emphasize the role of thyroid hormones in maintaining the normal functional state of the thymus.

CONCLUSIONS

Based on the research on the topic: "Modern Concepts of the Structural-Functional Interrelationships Between the Thymus and Thyroid Gland," the following conclusions are presented:

1. Pharmacologically reproducible experimental models of hypothyroidism (using mercazolil) and hyperthyroidism (by administering exogenous L-thyroxine) are the most physiological and adequate to thyroid dysfunctions observed in clinical practice.
2. Prolonged hypothyroidism during the prepubertal period is marked by a significant reduction in free T3 and T4 concentrations (over threefold), while thyroid-stimulating hormone (TSH) levels

increase fourfold compared to controls. Hypothyroidism induces pronounced thymic hypoplasia, resembling accidental involution of the organ. These changes manifest as:

1. A decrease in the thymus mass index, a reduction in the absolute areas of lobules and cortex, and a lower density of thymocyte distribution.
2. Thymic hypoplasia results from an imbalance between thymocyte proliferation and apoptosis—hypothyroidism suppresses cell proliferation while simultaneously enhancing cell death processes.

3. Short-term hypothyroidism, induced by cessation of the antithyroid drug Mercazolil, leads to a more moderate thymic hypoplasia, with a tendency to restore balance between thymocyte proliferation and destruction.
4. Therefore, timely elimination of thyroid hormone deficiency contributes to the restoration of the structural-functional parameters of the thymus.
5. Hyperthyroidism induced by exogenous L-thyroxine administration is characterized by increased T3 and T4 concentrations alongside decreased thyroid-stimulating hormone (TSH) levels. Prolonged hyperthyroidism leads to thymic hyperplasia, which manifests as:

1. A moderate increase in the thymus mass index, expansion of lobular areas due to cortical zone growth, and increased density of thymocytes and epithelial-reticular cells.
2. A rise in mitotically dividing cells, accompanied by a moderate increase in destructive cells.

6. Short-term hyperthyroidism, resulting from the cessation of exogenous L-thyroxine administration, presents with moderate thymic hyperplasia, showing a tendency toward normalization of the organ's structural and functional parameters.

7. Experimental hypo- and hyperthyroidism in adult animals produce thymic changes similar to those observed during the prepubertal period. However, these alterations are less pronounced compared to younger animals. This indicates that the immune system in the prepubertal period is significantly more sensitive to thyroid hormone imbalances, which exert a modulatory effect on the thymus and, consequently, on immune function as a whole.

Practical Recommendations

The findings highlight that the immune system—particularly its key organ, the thymus—is highly vulnerable to thyroid hormone imbalances during developmental phases in childhood and adolescence. Therefore, careful monitoring of thyroid function in young individuals is essential, especially in regions where iodine deficiency is prevalent.

When addressing hypothyroidism with synthetic thyroid hormones, it is important to manage hormone levels in the blood with great care. Excessive hormone levels could disrupt immune balance and lead to adverse effects on the immune system's stability.

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