



## **PATHOMORPHOLOGICAL FEATURES OF THYMUS IN INTRAUTERINE-INFECTED NEWBORNS WITH BODY HYPOTROPHY**

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### **Abstract**

In the work, an analysis of pathomorphological changes in the thymus of infants with extremely low body weight in general hypotrophy of organism (ENMT) developed under conditions of intrauterine infection was carried out. A study group included 77 body hypotrophy neonates who had developed in the presence of in utero infection. The main causes of their death were the following conditions: generalized viral and bacterial infection of mixed genesis (n=49 (63,6%)), congenital pneumonia (n=14 (18,2%)), bilateral hemorrhage into the ventricular system of the brain (n=12 (11,4%)), congenital sepsis (n=4 (5,2%)), and visceral malformations (n=10 (13%)). A comparison group consisted of 27 body hypotrophy (disorders of blood supply, dystrophy) babies; the main cause of their deaths was asphyxia resulting from acute uteroplacental circulatory disturbances. Transplacentally transmitted infections were not identified in this group. Thymic structural features in the examined groups were studied using a set of current morphological studies. Histological, immunohistochemical, electron microscopic and morphological studies revealed three variants of thymic structural changes: normoplastic, retardant and dysplastic. Anomalies of the shape, ectopia, and hypoplasia of the thymus, impaired corticomedullary differentiation in the lobules, and decreased CD1a, CD3 T-cell expression were shown to be the morphological signs of dyschronic development of the thymus. The morphological criteria for the retardant and dysplastic types of dyschronic thymic development were determined, which constitute the structural basis of immunodeficiency states in in utero infected ELBW newborn infants





**Keywords:** thymus, newborns, hypotrophy, extremely low body weight, thymus, developmental dyschronia, dysplasia, infection, sepsis.

## **Relevance**

In the fetus, the immune system, being one of the regulatory systems, provides a stable state of internal fetal homeostasis. Prolonged action of infectious agent is accompanied by depletion of compensatory, reserve capabilities of thymus and disorders of cellular-tissue differentiation of organ [9, 12]. At the same time, insufficient study of the immune system in fetuses and newborns of 22-27 weeks of gestation should be taken into account, as evidenced by few publications with rather contradictory information on structural and functional changes of immunogenesis organs [7, 8]. Individually, the immune system of fruits in the fetal period of ontogenesis is poorly studied. The obtained new knowledge will serve to diagnose the pathology of the immune system, primarily dyschronium or tissue malformations, which form the structural basis of immunodeficiency states [7.8]. The fetal immunogenesis system responds to the antigenic effects of the maternal organism, realizing its adaptation and compensatory capabilities. It has been found that such processes in the immune system as proliferation, differentiation, migration, cooperation and apoptosis are genetically deterministic [1,2]. The main component of the reticular framework of the thymus is polyfunctional epithelial cells. It has been proved that reduction of hormone-producing and cytokine functions of these cells, accompanied by their structural changes, is realized in the form of immunodeficiency states [5, 6]. One of the main reasons for the high rates of perinatal morbidity and mortality of children with hypotrophy (birth weight 500-950 g) are infectious diseases, the development and outcome of which depend on the morphofunctional maturity of the organs of the immune system of the fetus and newborn [10, 11]. At the same time, morpho immunogenesis is a complex process of interaction of progenitor cells and immature thymocytes with structural components of the stroma that form the microenvironment for lymphocytes [3, 4].

## **Target**

This study was an analysis of pathomorphological changes in the thymus of newborns with body hypotrophy that developed under conditions of intrauterine infection.

## **Material and Methods**

In the study of work, the main group was 77 newborns with body hypotrophy at birth. The death of 56 children occurred in the early, 21 - in the late neonatal periods. The





main causes of neonatal death were the following diseases: 49 (63.6%) patients died from generalized viral-bacterial infection of mixed genesis, 14 (18.2%) from congenital pneumonia, 12 (11.4%) from bilateral hemorrhages to the ventricular system of the brain, 4 (5.2%) from congenital sepsis and 10 (13%) children from malformations of internal organs. The etiology of inflammation was determined by direct and indirect immunofluorescence on swabs-fingerprints of autopsy material using diagnostic kits containing appropriate monoclonal antibodies labeled with fluorescein isothiocyanate (FITC). In the newborns of the main group, in 43.4% of cases, herpetic was detected, in 11.4% - chlamydia, in 34.7% - ureplasmic and in 7.5% - mycoplasmic infections, which in 42.3% of cases were combined with bacterial. The study of the structural features of thymus in the study groups was carried out using a complex of modern morphological research methods, including organometry, review histology, elective coloring methods (according to hematoxylin-eosin, van Gison, toluidine blue, PAS reaction), immunohistochemistry with determination of CD1a expression, CD3 T lymphocytes on frozen thymus sections, transmissima The comparison group combines thymuses from 30 deceased children with body hypotrophy, the main cause of death of which was asphyxia, which developed as a result of an acute disorder of utero-placental circulation. No infections transmitted transplacentally were detected in newborns of the comparison group. It should be emphasized that newborns of the main and comparison groups are comparable in terms of gestation dates (25-27 weeks). Immunohistochemical studies were carried out on sections of thymus tissue pieces fixed for 2 hours in a 4% paraformaldehyde solution and prepared on Microm NM cryostat. The sections with the primary antibodies were incubated for 18 hours at 4 ° C, with the secondary biotinylated antibodies in a wet chamber at room temperature for 20 minutes. Further, the sections were stained with diaminobenzidine, methyl green was used to identify the nuclei, and after dehydration, the sections were placed in balm. Lymphocyte differentiation was assessed with specific monoclonal antibodies to CD1a, CD2, CD3 T lymphocytes from "DakoCytomation." Evaluation of the results of immunohistochemical reactions was carried out by quantitative method according to the method we modified McCarthy et al. (2015). In ten microscope fields of view, at an increase of 400 times, 100 cells with different staining intensity were counted, which was estimated from 0 to 3 points. The expression index (IE) of the studied factors was determined by the formula:  $IE = \sum R(i) / 100$ , where  $i$  is the intensity of staining in scores from 0 to 3;  $P(i)$  is the percentage of cells stained at different intensities. In a number of cases, only qualitative assessment was used and, depending on the intensity of staining, it was estimated as negative (-), weak (+),





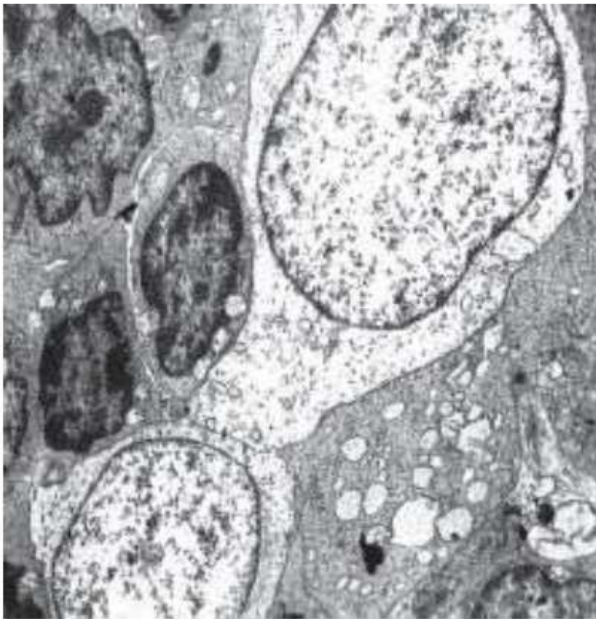


moderate (+ +) and sharply positive (+ + +). Evaluation of the quality of the reaction was carried out on sections with positive control for each of the antigens to be determined. Thymus samples for transmission electron microscopy were fixed in 2.5% glutaraldehyde solution. Post-fixation treatment of the material included double recovery of the fixer in 0.1 M Miloning buffer and additional post-fixation with 1% OsO<sub>4</sub> solution on phosphate buffer for 1-1.5 hours. Samples were dehydrated in solutions of ethanol and acetone of rising concentrations and enclosed in a mixture of Fluka epoxy resins. Semi-thin sections (1 μm) prepared on Leica Ultracut UCN were stained with methylene blue in combination with azure II and fuchsin base. Ultrathin sections were contrasted on the mesh with a saturated solution of uranyl acetate in 100% methyl alcohol prepared in admixture with lead nitrate. The thymus ultrastructure was studied in an electron transmission microscope EVM-100AK. The obtained digital material was processed by the methods of variation statistics using the Statistica software. The validity of the differences between the variation rows with the normal type of distribution was assessed by Student's test. The differences were considered valid at  $p < 0.06$ .

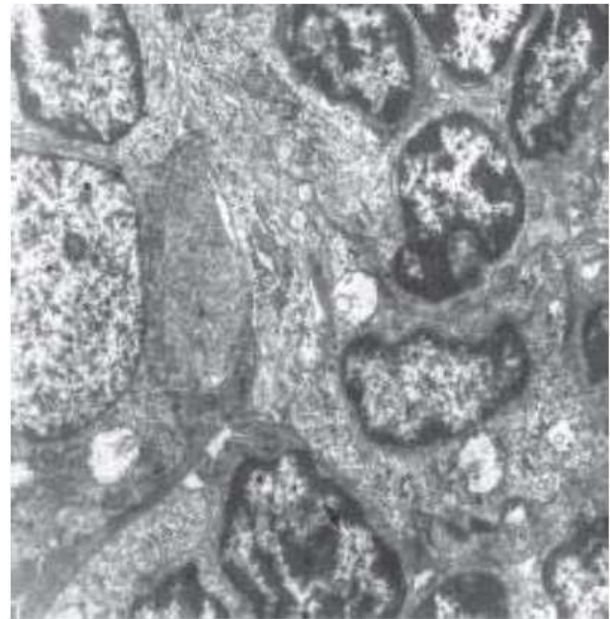
## Results and Discussion

When performing a comprehensive morphological analysis of thymus of 77 newborns of the main group obtained during preterm birth in 22-27 weeks, 3 variants of structural changes were revealed. Thymus 21 (18.2%) of the newborns of the main group had a typical form, was represented by two lobes connected at the base by an isthmus. In the subcapsular zone of the lobes, 2 types of epithelial cells were determined. Cells of the first type of elongated or triangular shape are located on a continuous basement membrane. Moderate chromatin marginalization in the nucleus, a developed granular endoplasmic reticulum and a large number of vacuoles with a protein substrate in the lumen are structural confirmation of the participation of these cells in hormone synthesis (Figure 1, a). The second type of reticulo epithelial cells (REC) has a star shape with a rounded nucleus with a diameter of 12 μm and finely condensed chromatin. Few tonophilaments, multivesicular bodies, vacuoles, short profiles of the rough endoplasmic network and a well-developed plate complex are visualized in the cytoplasm (see Fig. 1, b). Lymphocytes of the subcapsular zone are dominated by lymphoblasts expressing CD2 antigens on the membrane (IE 2.78 conditional units).

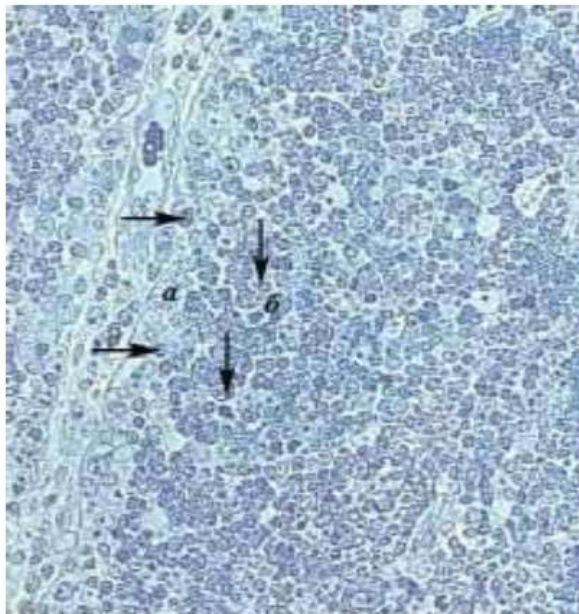




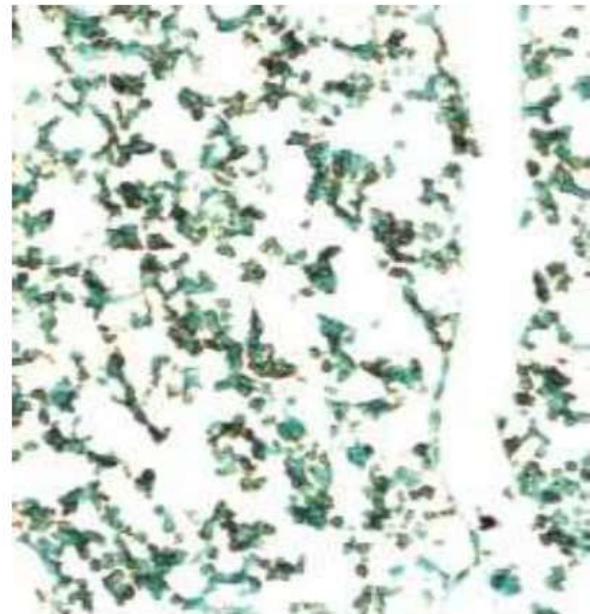
a  
A



b  
B



C



D

Figure 1. Morphological features of the normoplastic variant of thymus development in newborns with body hypotrophy. a - REC of the first type. Moderate marginalization of chromatin in the nucleus, vacuole with a protein substrate, invaginations of nucleolemma. TEM,  $\times 5000$ ; b - REC of the second type. Tonophils, multivesicular bodies, vacuoles, short profiles of a rough endoplasmic network and a developed plate complex. TEM,  $\times 5200$ ; c - in - EPRs of the first and second types ( $\rightarrow$ ), lymphoblasts; d - formation of lymphocytic-epithelial





"modules" (↓). Half-thin cut. Color methylene blue.  $\times 450$ ; d is expression of CD3 T lymphocytes. Immunoperoxidase method.  $\times 240$ .

In 71.8% of cases, the left share in linear parameters exceeded the right. The thymus was dominated by slices of medium and small sizes with twice the specific volume of cortical matter ( $65.4 \pm 0.4\%$ ) above the cerebral ( $34.4 \pm 0.8\%$ ). The inner cortical zone of the lobes of the thymus consists of a broadly loopy network of REK. Structural elements of this zone form peculiar lymphocytic-reticulo epithelial modules. In the center of these formations, light REC is determined, surrounded by medium and small lymphocytes (see Figure 1, c). Direct membrane contact of lymphocytes and REC is one of the main microenvironment factors determining thymocyte differentiation in the internal cortical zone. In this lobe area (68%), medium diameter lymphocytes ( $6-9 \mu\text{m}$ ) dominate, forming receptors to the antigens CD1, CD2, CD3 (see Figure 1, g). The specific volume of brain matter is 33.6%. Numerous processes of REK create a picture of loopy syncytium. Fan-like tonophilaments are visualized in the EPC cytoplasm. There is a redistribution of thymocytes with an increase of up to 68% of small forms. T-lymphocytes of the medullary zone, unlike cortical lymphocytes, are mostly mature phenotype and functionally active. Among them, T-lymphocytes with CD2, CD3 antigens predominate. Two types of REC are defined around mature thymic bodies. The first type is distinguished by a polygonal form with marginally condensed chromatin in the core and a large number of tonophilaments. A distinguishing feature of the second type of cells is the presence of large intracytoplasmic vacuoles. With this version of thymus development, Gassal bodies dominate, corresponding to the phase of morphofunctional maturity is the fineness of their contours, a seven-fold increase in the specific volume of interstitial tissue ( $17.34 \pm 0.31\%$ ) and a two-fold decrease in the parenchymal-stromal coefficient in them (2.4 units). Interstitial layers and thymus septa contain cellular infiltrates represented by macrophages with PAS-positive inclusions or a combination of macrophages with various granulocytogenesis cells (neutrophil leukocytes, eosinophils, basophils).

One of morphological evidence of lymphocyte differentiation impairment in the second variant of pathomorphological changes in thymus is a two-fold decrease in the density of light-type epithelial cells in the subcapsular zone of the lobes against the background of a reliable decrease in the specific volumes of both epithelial cells (11.7%) and lymphocytes (69%).

Among the epithelial cells of the outer layer there are areas of "deepithelization," the number and area of which increases in the lateral sections of the lobes. Cell elements are unevenly distributed in these zones. Areas of sharp thymocyte dilution alternate with group clusters of cells. The detected decrease in proliferative activity of





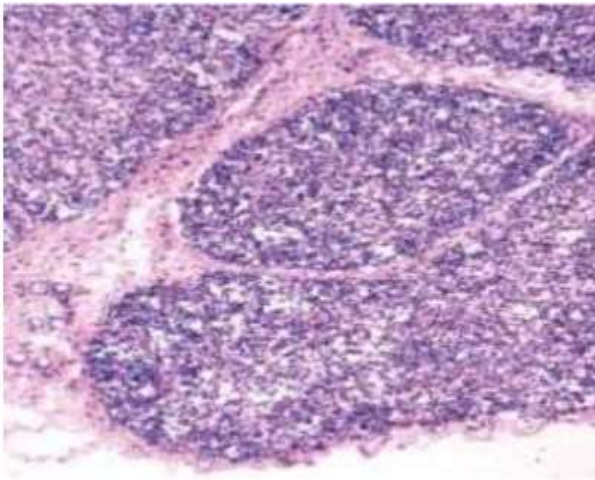
thymocytes is confirmed by reduction to  $0.39 \pm 0.03$  conditional units ( $p < 0.06$ ) of average histochemical coefficient of DNA in lymphocytes of subcapsular zone. Single lymphoblasts, large and small lymphocytes surround capillaries with a thickened basement membrane. Epithelial cells, located subcapsular, poorly express cytokeratin and contain small PAS-positive inclusions. Around isolated reticuloepitheliocytes, large lymphocytes and single (1-2) lymphoblasts are grouped in the form of a ring and a semi-ring. In the cytoplasm of REK, along with a decrease in the number of vacuoles, lysosomes, and the volume of the granular endoplasmic reticulum, the destruction of mitochondrial crystals progresses. Cell nuclei with submembrane localization of large-lobed heterochromatin. Kariolemma REK forms deep invaginations. Ultrastructural rearrangements in the REC are accompanied by a decrease in the CD1a expression index, CD3 T lymphocytes. In addition to epithelial cells and lymphocytes, single macrophages and mast cells are present in the subcapsular zone, and focal clusters of lipocytes occupying 1/- 1 are determined in the parenchyma. 8 10 diagnosed in thymus 49 (63.6%) of newborns with body hypotrophy, was distinguished by a reliable ( $p < 0.002$ ) decrease in the organ of metric parameters and thymico-mass coefficient, the value of which was 0.002 conditional units. In 64.4% of cases, atypical forms of thymus were found in the form of additional (2-3) lobes (28.4%) and hypoplasia of the left lobe (5.4%). The most significant decrease in specific volume (69.8%) and density of lymphocytes (27.7 cells) was found in the inner cortical zone of the lobes. Number of formed lymphocyte-epithelial modules is reduced, which is morphological confirmation of lymphocyte differentiation disorder in internal cortical zone of thymus lobes. The formation of structures such as a "continuous" ring around light epithelial cells by medium and large lymphocytes is disturbed. These structures are dominated by middle lymphocytes. Dark epithelial cells are located around capillaries in the form of foci. Along with epithelial cells, lymphocytes also adjoin the vessels. The dominant in the cerebral matter of the lobes of the thymus are epithelial cells. Hypertrophied light-type epithelial cells are involved in the formation of Gassal bodies. Two types of epithelial cells are located around the forming thymic bodies. Cells of the first type with a light cytoplasm, with a rounded nucleus with a diameter of up to  $15 \mu\text{m}$  and focal marginalization of chromatin in German. A distinctive morphological feature of epithelial cells of the second type is the detection of vacuoles in the cytoplasm indicating the preservation of secretory function of this cell type. Gassal's bodies are characterized by quantitative, qualitative and topographic variability. In the second version of thymus development disturbance, single thymic bodies contact with basement membrane of capillaries and post-capillary venules. In the brain substance, the specific proportion



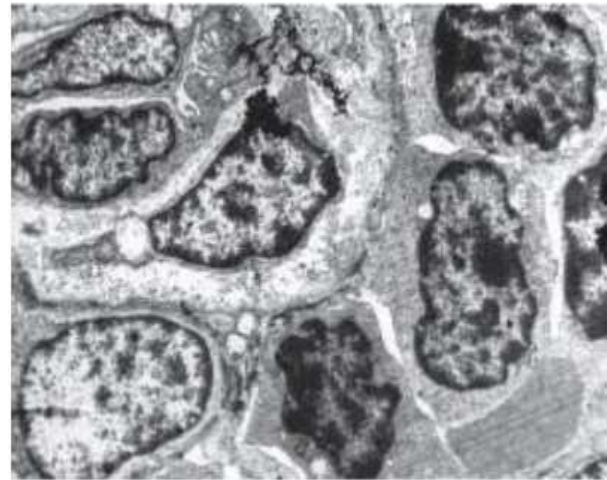




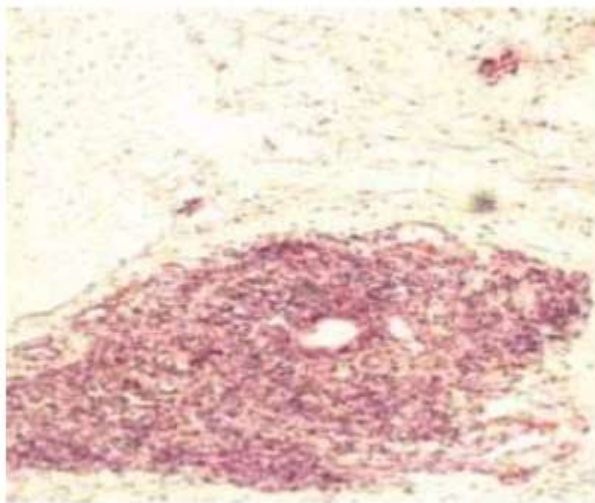
of young bodies was reduced by 1.5 times against the background of an increase to 36.3% in the number of cystic-transformed Gassal bodies surrounded by a fibrous capsule. In the center of the latter, pulverized petrificates and fragments of REK, lymphocytes and neutrophilic leukocytes are determined. Due to the increased formation of keratogyalin, the diameter of individual thymic bodies increases and reaches 46-52 microns.



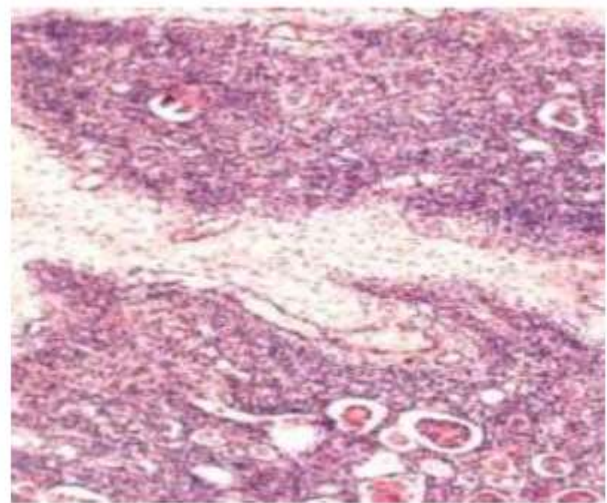
a



b



c



d

Figure 2. Pathomorphological signs of dyschronia of thymus development in newborns with body hypotrophy.





A - retardation of zonal differentiation in the lobes of the thymus. Coloring with hematoxylin and eosin.  $\times 240$ ; b - perivenular localization of single EPRs and middle lymphocytes. TEM,  $\times 8500$ ; c - thymus slices of small size without zonal differentiation, wide layers of connective tissue. Hypo-, delimitation of lobes. Coloring with hematoxylin and eosin.  $\times 150$ ; d - hypoplasia of thymus lobes. Cystic transformation and migration of thymic bodies to the periphery of the lobes. Color hematoxylin and eosin.  $\times 240$ .

Against the background of a sharp quantitative decrease in lymphocytes in the cerebral matter of the thymic lobes, a small number of macrophages and interdigitating U 18 (17.1%) of newborns with body hypotrophy were diagnosed with a third version of pathomorphological changes in the thymus. The central organ of the immune system in 63.6% of cases is abnormal in form with atypical localization. From the anomalies of the shape of the organ, such deviations from the variants of the norm as trefoil, hypoplasia of the right lobe and the presence of hypoplasized lobes at the base of the organ were revealed. Three children were diagnosed with ectopia of thyroid tissue. Anomalies of the form were combined with a reliable decrease in the organ of the metric parameters of the thymus mainly due to a decrease in the volume of parenchyma ( $54.23 \pm 0.37\%$ ;  $p < 0,06$ ). From parenchyma, rounded or oval-shaped small slices with a diameter of 74 to 113 microns are formed in hypoplasized timuses. Structural features of the thyroid gland in the third subgroup include violation of corticomedullary differentiation, a reliable decrease in the number of lymphocytes in the internal cortical (63.6%) and two-fold in the medullary (36.4%) regions of the lobes. Quantitative reduction of lymphocytes in thymus lobes is combined with a two-fold decrease in their density per unit area. The density of cells in the subcapsular zone was 14.8, in the internal cortical zone - 24.6 cells. The number of lymphocytes in the cerebral matter of the lobes ranged from 6 to 27 cells. T-lymphocytes are absent in the subcapsular zone, which indicates impaired migration of lymphocyte precursors from the red bone marrow.

The result of delayed and incomplete lobe formation is a decrease in the specific volume of septa ( $2.27 \pm 0.15\%$ ) with the latter located only within the subcapsular zone. In this embodiment, thymus dyschronium increases the specific volume of the interdigital connective tissue to  $29.94 \pm 0.34\%$ , which is 16 times higher than the same indicator in the comparison group (see Figure 2, c). It should be emphasized that the quadruple (1.29 conventional units) decrease in the parenchymal-stromal coefficient in the third variant of the thymus tissue differentiation disorder is due not only to an increase in the stromal component, but also to a decrease in the volume of the parenchyma. In the septa and interstitial tissue of the thyroid gland, scarce



macrophage infiltration. In the composition of infiltrates, single granulocytopoies cells are found. Disorders of tissue differentiation of vessels located in interstitial tissue are manifested by hypoplasia of muscle and elastic structures in the wall of stenosed arteries. The venous channel is expanded, especially in the areas of localization of full-blooded thin-walled venous collectors. In 38.7% of cases, focal clusters of lipocytes adjacent to the lobes of the thymus are visualized in the intermediate tissue. Fatty transformation of parenchyma of subcapsular zones of thymus lobes was diagnosed in 28.6% of cases. The shape, volume, diameter and topographic localization of Gassal bodies in the timuses of the third subgroup were characterized by variability. The specific volume of thymic bodies was  $2.63 \pm 0.09\%$ . Parameters compared to control group with downward trend. The average diameter of Gassal bodies ranged from 36 to 49  $\mu\text{m}$  and averaged 36.9  $\mu\text{m}$ . The increase in the diameter of the thymic bodies is associated with their cystic transformation and the formation of a fibrous-type capsule around the bodies. In single cases, combining 2-3 bodies into a single formation led to an increase in their diameter from 470 to 730 microns. In addition to brain matter, Gassal bodies are located in the peripheral parts of the lobes (see Figure 2, g). Such localization of thymic bodies is, in our opinion, associated with the functional-structural depletion of REC, which make up the cellular microenvironment and provide differentiation of lymphocytes in the subcapsular zone. The ratio of Gassal bodies differing in maturity varies towards a decrease in the specific volume of young bodies (15.4%) and mature (37.7%) types. At the same time, the specific volume of bodies in the regression stage, i.e., Gassal bodies of the old type, increases twice (46.9%). Structurally, such bodies have the following features: fibrous capsule, keratogialine, pulverized petrificates and fragments of nuclei. Among the old-type bodies described above, 76% are cystic-transformed forms.

## Conclusion

Thus, in the thymus of newborns with hypotrophy of the body, developed intrauterine in the conditions of infection, three options for structural changes were revealed: normoplastic, non-standard and dysplastic. Additional morphological features of the retrograde type of thymus dyschronia are a reliable increase in the specific volume of interstitial tissue to  $6.13 \pm 0.21\%$ , a low content of glycogen and neutral mucopolysaccharides in the medial and basal membranes of large vessels. The normoplastic variant of thymus development is characterized by domination of lobes with completed zonal differentiation, quantitative increase of Gassal bodies of young and mature types and predominance in the internal cortical zone of middle forms of lymphocytes forming receptors to antigens CD1a, CD2, CD3. Significant ( $p < 0.06$ )







reduction of thymus metric parameters organ with reduction of lobe diameter to 36 mm or less, preemption of specific volume of cortical substance (58.64%) over cerebral one, absence of corticomedullary differentiation in 37.8% of lobes, reduction to 69.8% of specific volume of lymphocytes, as well as CD1a expression index, CD3 T-lymphocytes and formed lymphocytic-epitol.

Confirmation of hormonal insufficiency and disruption of the cellular microenvironment of thymocytes in the retrograde version of thymus dyschronia is a two-fold decrease in the density of REC location, a decrease in the number of vacuoles and the specific volume of the granular endoplasmic reticulum against the background of mitochondrial destruction, as well as a decrease in the number and size of Gassal bodies (less than 75  $\mu\text{m}$ ). The absence of corticomedullary differentiation in the lobes, a reliable decrease in the number and density of thymocytes in all morphofunctional zones, cystic transformation of Gassal bodies against the background of a reliable decrease in their specific volume are structural confirmation of impaired proliferation and differentiation of thymocytes and depletion of the compensatory capabilities of their cellular environment. At the same time, the bulk of the bodies corresponds to the phase of morphofunctional maturity. Diagnostic morphological features of the dysplastic variant of thymus development include form abnormalities (74.8%), ectopia and organ hypoplasia, a two-fold decrease in the parenchymal-stromal coefficient due to the predominance of small lobes surrounded by wide layers of immature connective tissue.

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