

**O.V. Fedosieieva****IMMUNE-HISTOCHEMICAL INDICATORS  
OF THE MORPHO-FUNCTIONAL STATE  
OF THE THYROID GLAND IN NORM  
AND AFTER PRENATAL  
ANTIGENIC EXPOSURE**

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**Ключові слова:** тироглобулін, щитоподібна залоза, щури, антиген

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**Abstract.** Immune-histochemical indicators of the morpho-functional state of the thyroid gland in norm and after prenatal antigenic exposure. Fedosieieva O.V. During the individual development and exposure to infectious agents of the organism, the structural components of the thyroid gland can change significantly. In modern research, great importance is attracted to the study of mechanisms for maintaining morphological homeostasis of the thyroid gland and ideas about its structural and functional restructuring in response to the body's immune system to various infections. The morpho-functional features of the thyroid gland of newborn animals after prenatal action of thyroid-nonspecific antigen (staphylococcal toxoid) have been experimentally established. In antigen premiu rats, the timing of folliculogenesis changes, as well as the sizes, distribution and immunohistochemical expression of antibodies to thyroglobulin, the functional state of thyrocytes and follicles compared to normal change. The detected immuno-histochemical changes indicate signs of morpho-functional immaturity of the organ with hypoactivity elements.

**Реферат.** Иммуногистохимические показатели морфофункционального состояния щитовидной железы в норме и после пренатальной антигенной нагрузки. Федосеева О.В. В ходе индивидуального развития и влияния инфекционных агентов на организм структурные компоненты щитовидной железы могут существенно меняться. В современных исследованиях большое значение придается изучению механизмов поддержания морфологического гомеостаза щитовидной железы и представлений о ее структурно-функциональной перестройке в условиях реакции иммунной системы организма на различные инфекции. Экспериментально установлены морфофункциональные особенности щитовидной железы новорожденных животных после пренатального действия тиреоид-неспецифического антигена (стафилококкового анатоксина). У антиген-премированных крыс изменяются сроки фолликулогенеза, а также размеры, распределение и иммуногистохимическая экспрессия антител к тироглобулину, функциональное состояние тироцитов и фолликулов по сравнению с нормой. Обнаруженные иммуногистохимические изменения свидетельствуют о признаках морфофункциональной незрелости органа с элементами гипоактивности.

The thyroid gland is characterized by high structural and functional lability in response to the influence of various exogenous factors. In the course of individual development and influence of infectious agents on the body, the structural components of the thyroid gland can change significantly [4, 12]. In modern research, great importance is attracted to the study of mechanisms

for maintaining morphological homeostasis of the thyroid gland and ideas about its structural and functional restructuring in terms of the response of the body's immune system to various infections. Particular attention in this issue is drawn to the influence of various factors on the body of the pregnant woman, i.e. prenatal nonspecific action of external infectious antigens on the body, which

would have long-term manifestations after birth [5]. Antigenic load at critical times of ontogenesis can cause significant "breakages" in the child's immune system [10]. The consequence of the past infections can be the initiation of morphological changes of organs and barrier structures, i.e. these infections may not lead to permanent changes in the structure, but only leave a permanent immunological "background" in the organ and the body as a whole, which under the impact of various causative agents may further initiate pathological conditions, including autoimmune diseases, etc. [10, 15]. The peculiarities of the interaction of the fetus or newborn with exogenous antigens may be crucial in the formation of their immune status in the future [7, 8, 9, 13]. Under the influence of prenatal antigenic load there is a premature exit of T-lymphocytes from the thymus which in tissues change not only terms of development of structural elements, but also their immunological tolerance, etc. [1, 2, 3]. A number of scientific studies have shown that bacterial infection can provide both antigen-specific signal and antigen-nonspecific support required to induce a pathogenic autoimmune response [6, 11, 14]. Therefore, the study of thyroid morphogenesis under the conditions of prenatal action of antigens of infectious origin is a relevant and promising scientific direction.

The aim of the study was to establish the morpho-functional features of the thyroid gland of experimental animals in the norm and after prenatal action of thyroid-nonspecific antigen.

#### MATERIALS AND METHODS OF RESEARCH

The material for the study was the thyroid gland of Wistar rats aged 1 to 7 days of postnatal development (54 animals), 6 animals in each group. Three groups of animals on day 1, 3, 7 of life after birth were studied: I group – intact animals (norm); II group - control, animals which were injected with 0.9% NaCl solution on day 18 of the dated pregnancy; III group – experimental animals, which were injected with liquid purified adsorbed staphylococcal toxoid (10-14 binding units in 1 ml, diluted 10 times) on day 18 of dated pregnancy by the method of Voloshin MA. (Pat. 49377, Ukraine, 2010 and Pat. 63020, Ukraine, 2011). Antigen and 0.9% NaCl solution were administered to the fetus surgically during laparotomy, by intrauterine, transdermal subcutaneous injection into the interscapular area at a dose of 0.05 ml per fetus.

The thyroid gland was fixed in a 10% solution of neutral buffered formalin during the day. The objects were put into paraffin blocks by the conventional method. Histological sections 3-5  $\mu$ m

thick were stained with hematoxylin and eosin for survey light microscopy and morphometry.

Immunohistochemical study was used to establish synthetic activity, assess the functional state of the organ and performed in accordance with the protocol recommended by the manufacturer for a particular antibody. Thyroglobulin Antibody (2H11) from Santa Cruz Biotechnology, Inc. was used by the method of indirect staining with immunoperoxidase using conjugated HRP murine IgG-binding proteins, m-IgG $\kappa$  BP-HRP, followed by incubation in a substrate of peroxidase and a mixture of chromogen DAB-3-diaminobenzidine tetrachloride and by counterstaining of nuclei with Mayer hematoxylin, dehydration, brightening, and balming.

The result was regarded as positive in the case of chromogen salts in the form of a specific reaction (cytoplasmic, membrane reaction depending on the location of the antigen). The intensity of benzidine label deposition was evaluated in points according to the following gradation: "0" – no reaction, light yellow color. "1" – weak reaction, light brown color; "2" – moderate reaction, brown color; "3" – intense reaction, dark brown color. Intermediate shades were denoted as 0.5; 1.5 and 2.5 points, respectively. In order to control the method, a series of studies was conducted using positive and negative samples, which served as standards.

Photo documentation of the studied objects was performed using a Carl Zeiss microscope "Primo Star" using an AxioCam camera, a set of morphometric studies was performed using the Zeiss Zen program (2017).

Statistical analysis of the results was performed using a personal computer based on the Windows XP operating system using the statistical package "Statistica for Windows 6.0" (StatSoftInc., Serial number XAXXR712D833214FAN5), Excel (Microsoft Office, USA). Methods of variation statistics were used. All research results were recorded in journals and protocols of primary documentation, as well as with the use of electronic media. Significance of differences between groups was assessed using Student-Fisher t-test for a confidence level of at least 95% ( $p < 0.05$ ).

#### RESULTS AND DISCUSSION

Light microscopy of serial sections of the thyroid gland of animals of intact and control groups of the 1st day of life revealed that the parenchyma of the organ consists of follicles of colloidal-free type of secretion and interfollicular thyrocytes. The cavity in the center of most follicles is not visualized or the formation of the primary cavity begins (Fig. 1, a). At the same time, in prenatally antigen premium

animals of the experimental group, the glandular component of the thyroid gland is morphologically more structured: most follicles have a cavity with oxyphilic stained content without marginal vacuolation. Some follicles have an irregular shape and invaginations from thyrocytes into the lumen of the follicles, indicating continued folliculogenesis. The size of the thyroid follicles of animals of the experimental group was 1.3 times significantly larger compared to the control and intact groups, the data of which did not differ significantly. The expression of antibodies to thyroglobulin (Tg) in the thyroid gland of animals of group I and II is moderate and is visualized in the cytoplasm of thyrocytes. Cytoplasmic Tg<sup>+</sup>-expression is uneven and has a foamy appearance (Fig. 1, a), due to Tg<sup>-</sup> vacuoles, while in most follicular thyrocytes, prenatally antigen premium thyroglobulin is not detected immunohistochemically. In Tg<sup>+</sup> thyrocytes, the expression of the corresponding marker is weak and evenly diffusely distributed in the cytoplasm (Fig. 1, b). Moreover, in the hollow follicles of the experimental group thyroglobulin was not detected immunohistochemically (Fig. 1, b). The number of Tg<sup>+</sup> thyrocytes of animals of group III is 2.4 times less per unit of area compared to groups I and II.

On the 3rd day of postnatal life in the thyroid gland of animals of groups I and II, single hollow follicles with colloidal type of secretion appear, which are mostly subcapsular. Among the spherical follicles the part is of the irregular shape. Cubic follicular thyrocytes lie on the basement membrane, tightly connected to each other. Moderate, sometimes intensive cytoplasmic Tg<sup>+</sup> expression of follicular thyrocytes is revealed. Immunohistochemically, thyroglobulin with intensive parietal expression and moderate central thyroglobulin is detected in the follicle cavity. Small marginal vacuoles of colloid resorption are partially visualized in single follicles of medium diameter. During this period in the thyroid gland of experimental animals, follicles of hollow colloidal type appear throughout the volume of the organ. Follicles of medium diameter of spherical shape are localized under the capsule. The intensity of Tg<sup>+</sup> expression is weak and uniform throughout the follicle cavity, even parietal in the area of resorption vacuoles. Cytoplasmic Tg<sup>+</sup>-reaction is weak but uniform in all follicular thyrocytes.

The ratio of follicles of different sizes (small: medium: large) in animals of the intact and control groups on the 7th day is 3:1:0, and in antigen premium – 1:2:1, respectively. In the ratio of tissue components a decrease in the specific area of the thyroid epithelium per unit of area is revealed. This

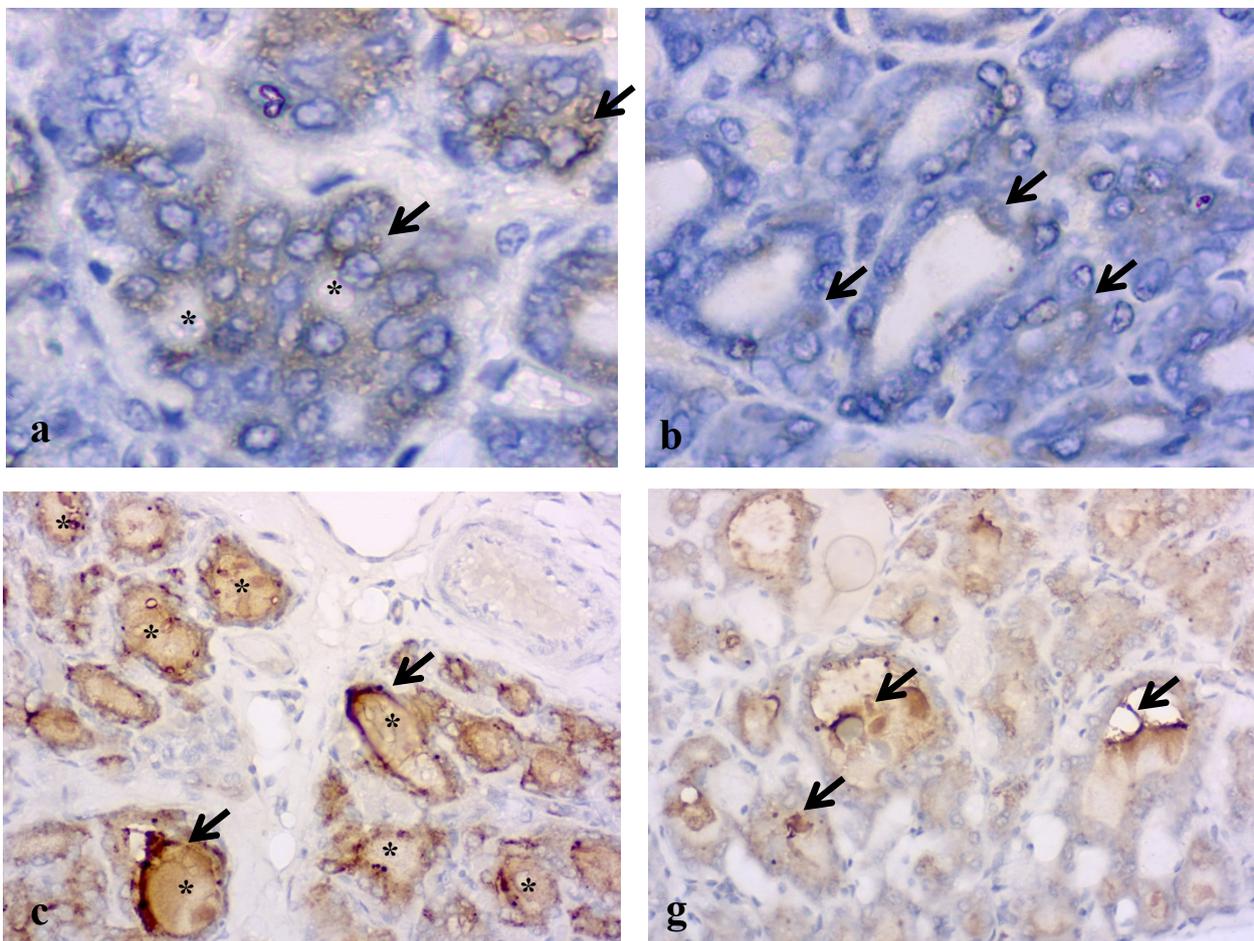
is due to the fact that the height of the cells of the follicular epithelium becomes smaller, cubic and flat shape of thyrocytes predominates, cylindrical cells occur rare, mainly in small follicles of colloidal type. The average diameter of the nuclei is significantly reduced compared to the control group, being  $0.25 \pm 0.01 \mu\text{m}$ . The number of nucleoli in thyrocytes per unit of area in the experimental group significantly decreased, as compared with the control, which is  $22.7 \pm 1.04$  and  $34.4 \pm 1.01$ , respectively, indicating a decrease in the synthesis of subunits of ribosomes, and hence gross synthesis of protein products. An increase in the relative percentage of the area of the follicle cavity due to an increase in the number of large and medium follicles in antigen premium animals compared with groups I and II was revealed. The absence of resorption vacuoles in a number of follicles respectively is accompanied by a delayed excretion of hormones into the bloodstream and leads to overstretching of such follicles and, as a consequence, flattening of the thyroid epithelium. This is fully confirmed immunohistochemically by the presence of weakly expressed Tg<sup>+</sup> expression both in the cytoplasm of thyrocytes and moderate in the colloid of thyroid follicles of antigen premium rats (Fig. 1, d), in contrast to animals of intact and control groups, where the reaction was intense in these structures (Fig. 1, c). Tg<sup>+</sup> colloid with signs of degeneration was also detected in large thyroid follicles of experimental animals (Fig. 1, d).

Thus, in the neonatal period, rats undergo significant morpho-functional changes in the parenchyma of the thyroid gland, which are characterized by intensive folliculogenesis and intracellular development of the synthetic apparatus of thyrocytes, which is characterized by pronounced production of prehormone (thyroglobulin) and hormones of the thyroid gland. It has been experimentally proven that in prenatally antigen premium animals with staphylococcal toxoid anatoxin, the timing of formation of structural and functional units of the thyroid gland, their size, distribution and functional state change, as compared to normal. Despite a more developed morphological structure of the thyroid gland and the advance of folliculogenesis compared with intact animals, the experimental group showed immunohistochemical signs of functional immaturity of the organ, which is characterized by weak cytoplasmic and colloidal Tg<sup>+</sup> expression. This is a sign of reduced protein-synthesizing function of thyrocytes, and almost absence of vacuoles of resorption and flattening of the thyroid epithelium of individual follicles are morphological signs of reduced

hormone-producing function of the thyroid gland. Moreover, such a morphological picture was visualized on serial sections in both lobes throughout the parenchyma of the organ and being not mosaic, which allows us to assert the signs of

morphofunctional immaturity of the organ with signs of hypoactivity.

Conflict of interest. The author declares no conflict of interest.



**Immunohistochemical expression of antibodies to thyroglobulin in the thyroid gland of newborn rats of Wistar line on day 1 (a, b) and 7 (c, d) of life. Magnification: a, b – x1000; in, g – x400.**

**a** – weak cytoplasmic expression of Tg<sup>+</sup> in thyrocytes of colloid-type follicles of the thyroid gland of rats of the control group (indicated by arrows); asterisks indicate the formation of the primary cavity of the follicles.

**b** – arrows indicate Tg<sup>+</sup> thyrocytes with weakly expressed immunohistochemical expression of antibodies of hollow thyroid follicles of antigen premium animals of the experimental group.

**c** – thyroid gland of animals of the control group, arrows indicate Tg<sup>+</sup> follicles with intensive cytoplasmic and colloidal expression, asterisks indicate colloid with resorption vacuoles.

**g** – arrows indicate a colloid with signs of degeneration of follicles of the thyroid gland of animals after prenatal antigenic loading with staphylococcal anatoxin.

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