

Реферати

**ПОКАЗНИКИ КЛІТИННОГО ЦИКЛУ
В ЩИТОПОДІБНІЙ ЗАЛОЗІ ПІСЛЯ
ТЕРМІЧНОГО ОПІКУ ШКІРИ ПРИ
ЗАСТОСУВАННІ РОЗЧИНІВ ЛАКТОПРОТЕІНУ
З СОРБИТОЛОМ АБО HAES-LX 5 %**

**Тирон О.І., Аппельханс О.Л., Гунас В.І.,
Черешнюк І.Л., Лисенко Д.А.**

Вміст ДНК в ядрах клітин щитоподібної залози 90 білих шурів-самців на фоні опіку шкіри 2-3 ступеня (із площею ураження 21-23 % поверхні тіла) і введення розчинів лактопротеїну з сорбітолом або HAES-LX 5 % визначали методом проточної цитометрії. Через 1, 3, 7 та 14 діб після термічної травми шкіри і застосування лактопротеїну з сорбітолом або HAES-LX 5 % встановлено лише менші значення показників S-фази у порівнянні із показниками груп без опіку. Через 21 добу після термічного ушкодження шкіри в групі з інфузією HAES-LX 5 % показник інтервалу SUB-G0G1 суттєво більший порівняно з аналогічним показником контрольної групи. Через 30 діб в групах з попереднім введенням розчинів HAES-LX 5 % та лактопротеїну з сорбітолом величина показнику SUB-G0G1 значно більша від аналогічного в групах без опіку шкіри.

Ключові слова: щитоподібна залоза, термічний опік шкіри, ДНК-цитометрія, HAES-LX 5 %, лактопротеїн із сорбітолом.

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**ПОКАЗАТЕЛИ КЛЕТОЧНОГО ЦИКЛА
В ЩИТОВИДНІЙ ЖЕЛЕЗЕ ПОСЛЕ
ТЕРМИЧЕСКОГО ОЖОГА КОЖИ ПРИ
ПРИМЕНЕНИИ РАСТВОРОВ ЛАКТОПРОТЕИНА
С СОРБИТОЛОМ ИЛИ HAES-LX 5 %**

**Тирон О.И., Аппельханс Е.Л., Гунас В.И.,
Черешнюк И.Л., Лысенко Д.А.**

Содержание ДНК в ядрах клеток щитовидной железы 90 белых крыс-самцов на фоне ожога кожи 2-3 степени (с площадью поражения 21-23 % поверхности тела) и введение растворов лактопротеина с сорбитолом или HAES-LX 5 % определяли методом проточной цитометрии. Через 1, 3, 7 и 14 суток после термической травмы кожи и применения лактопротеина с сорбитолом или HAES-LX 5 % установлено только меньшие значения показателей S-фазы по сравнению с показателями групп без ожога. Через 21 день после термического повреждения кожи в группе с инфузией HAES-LX 5 % показатель интервала SUB-G0G1 существенно больше по сравнению с аналогичным показателем контрольной группы. Через 30 суток в группах с предварительным введением растворов HAES-LX 5 % и лактопротеина с сорбитолом величина показателя SUB-G0G1 значительно больше аналогичного в группах без ожога кожи.

Ключевые слова: щитовидная железа, термический ожог кожи, ДНК-цитометрия, HAES-LX 5 %, лактопротеин с сорбитолом.

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**MORPHOGENESIS OF RAT'S THYROID GLAND IN PREWEANING PERIOD AFTER
PRENATAL INFLUENCE OF STAPHYLOCOCCAL TOXOID**

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The results were obtained about morphogenesis of rat's thyroid after intrauterine antigenic action of staphylococcal toxoid. Prenatal influence of staphylococcal toxoid led to the formation of a more pronounced structure of the parenchyma and stroma, but they showed signs of functional immaturity, which led to the presence of a morphological picture of hypothyroidism after birth (rats 1-7 days of postnatal ontogeny). With the beginning of the middle sucking period (7-21 days of life) there is a lymphoid infiltration in separate sites of a thyroid gland, processes of reorganization of the synthetic device and resorption of colloid, so functional "maturation" of already morphologically formed structures begins. Such abrupt changes in the thyroid gland of experimental animals are due to systemic prenatal antigenic influence on the body as a whole and is adaptive-compensatory in nature.

Keywords: thyroid gland, antigen, staphylococcal toxoid, morphogenesis, experiment.

The work is a fragment of the research project "Immunomorphological features of internal organs under the effect of endogenous and exogenous factors on the body", state registration No. 0118U004250.

The current stage of the morphology development is characterized by increasing interest to the morphological and functional components of tissues, organs and their systems, that's why the issue of dynamic morphology is gaining more and more recognition. The thyroid gland (thyroid), is a heterogeneous tissue complex system [2, 6]. Structural and functional alteration of thyrocytes in various pathological conditions is a topical problem of biology and medicine, because thyroid hormones influence to the numerous processes of the vital activity in the body. Many authors note lability in morphology and functional activity of the thyroid gland in response to various aggressive factors of both exogenous and endogenous nature [2, 4, 6]. Antigenic influence at critical terms of ontogenesis can cause significant changes in the child's immune system. It is known that the entry of antigens into the fetus causes premature release of T-lymphocytes from the thymus and their migration to various organs. In these organs the tempo and terms change in formation of the basic structural components [1, 3, 5]. The appearance of more "aggressive" bacterial and viral pathogens led to a significant increase in the number of thyroid pathology [7, 8]. The thyroid gland, like every other organ, is characterized by a specific algorithm of functioning, which has the appropriate morphological design in the form of a hierarchy of cellular, tissue and organ units [2, 4, 6, 8].

The problem of infections caused by gram-positive microorganisms is growing steadily around the world. Staphylococci are the causative agents of much of the pneumonia, infections of the skin and soft tissues, bones and articulations, sepsis, etc. Staphylococcal infection poses the greatest threat to newborns and children in the first months of life. Therefore, in order to reduce the incidence among them, there are active immunization of pregnant women "at risk" with staphylococcal toxoid at 32, 34 and 36 weeks of pregnancy. Structural and functional changes, reactivity of structural and functional units, cellular and non-cellular components of the thyroid during postnatal ontogenesis in norm and after intrauterine action of staphylococcal toxoid have not been studied yet. Therefore, we consider the study relevant, given its practical significance and direction.

The purpose of the work was to study the features of rat thyroid gland's morphogenesis in suckling period at norm and after prenatal influence of staphylococcal toxoid.

Materials and methods. The material for the study was the thyroid gland of Wistar rats aged 1 to 21 days of postnatal development (108 animals), about 6 animals in each group. Three animal groups were studied on days 1, 3, 7, 11, 14, 21 after birth. Group I included intact animals (norm); group II was the control, animals which were injected intrauterine 0.9% NaCl solution; group III consisted of experimental animals injected with staphylococcal toxoid liquid purified adsorbed (10-14 units of binding in 1 ml, diluted 10 portions) intrauterine, on the 18th day of dated pregnancy by the method of Voloshin MA (Pat. 49377, Ukraine, 2010 and Pat. 63020, Ukraine, 2011). Injections of antigen or 0.9% NaCl solution for fetus were performed surgically during laparotomy, by intrauterine, transdermal subcutaneous ways at the dose of 0.05 ml to each fetus. The distribution of the material is presented in table 1.

Table 1

Distribution of material under study

Age groups of the suckling period	Day of a life	Intact group	Control group	Experimental group
Newborns (early suckling)	1	6	6	6
	3	6	6	6
	7	6	6	6
Suckling (middle suckling)	11	6	6	6
	14	6	6	6
	21	6	6	6
Total		36	36	36

The thyroid gland was fixed in a 10% solution of neutral buffered formalin during the day. The objects were filled into paraffin blocks by the conventional method. Histological sections 3-5 μ m thick were stained by hematoxylin-eosin, histochemically by PAS reaction, by Van Gizon staining, by azure-II-eosin. A set of morphometric studies was performed by microscope Carl Zeiss Primo Star equipped with the Axiocam digital microphoto attachment with using program complex Zeiss Zen 2011. The results were considered reliable at $p < 0.05$. To summarize the digital material, for processing of statistical material the standard software packages were used: Microsoft Office Excel and Statistica 10.0.

Results of the study and their discussion. In serial sections of the animals' thyroid in the intact and control groups from 1st day of life at early suckling period it was proved, that parenchyma is characterized by the presence of numerous globular clusters of thyrocytes - follicles without colloid, which are closely neighboring upon each other (fig. 1a).

Thyrocytes have cuboidal form, they are not yet clearly distinguished by apical and basal poles. The nuclei contain 1-3 nucleoli, which indicates a protein-synthetic activity. The basement membrane is not visualized, so these globular formations have the appearance of a chaotic cell mass, but not organized structures. On the 3rd day, single follicles occur with colloidal type of secretion, which are located mainly under the thyroid capsule (fig. 1.b).

In animals of early suckling period in intact and control groups the thyroid stroma is weakly expressed, the capillary network is not clearly visualized between the follicles, but the thyroid parenchyma is divided into lobes by layers of connective tissue and contains vessels and nerves.

The microscopic study of serial sections of a thyroid gland in experimental group (prenatally antigen-awarded animals) at the age of 1-7 days, attention is drawn by recalibration of arteries, expansion of capillaries, increase in a stromal component between follicles of colloidal type in the thyroid gland. During this period, colloidal follicles occur throughout the thyroid, but mostly under the capsule, in contrast to animals of the intact and control groups.

In animals of the intact and control groups on the 7th day, the ratio of follicles of different sizes (small: medium: large) is 3: 1: 0, and in antigen-premium - 1: 2: 1, respectively. In the ratio of tissue components a decrease was revealed in the specific area of the thyroid epithelium. This is due to the fact that the height of the cells of the follicular epithelium becomes smaller, the cubic and flat form of thyrocytes predominates, cylindrical cells are rare, mainly in adenomers of small size of the noncolloid type. An increase in the relative percentage of the area of the colloid due to an increase in the number of large and

medium-sized follicles, which contain dense colloid and desquamated cells (fig. 1b). Boundary vacuolation of the colloid is not detected.

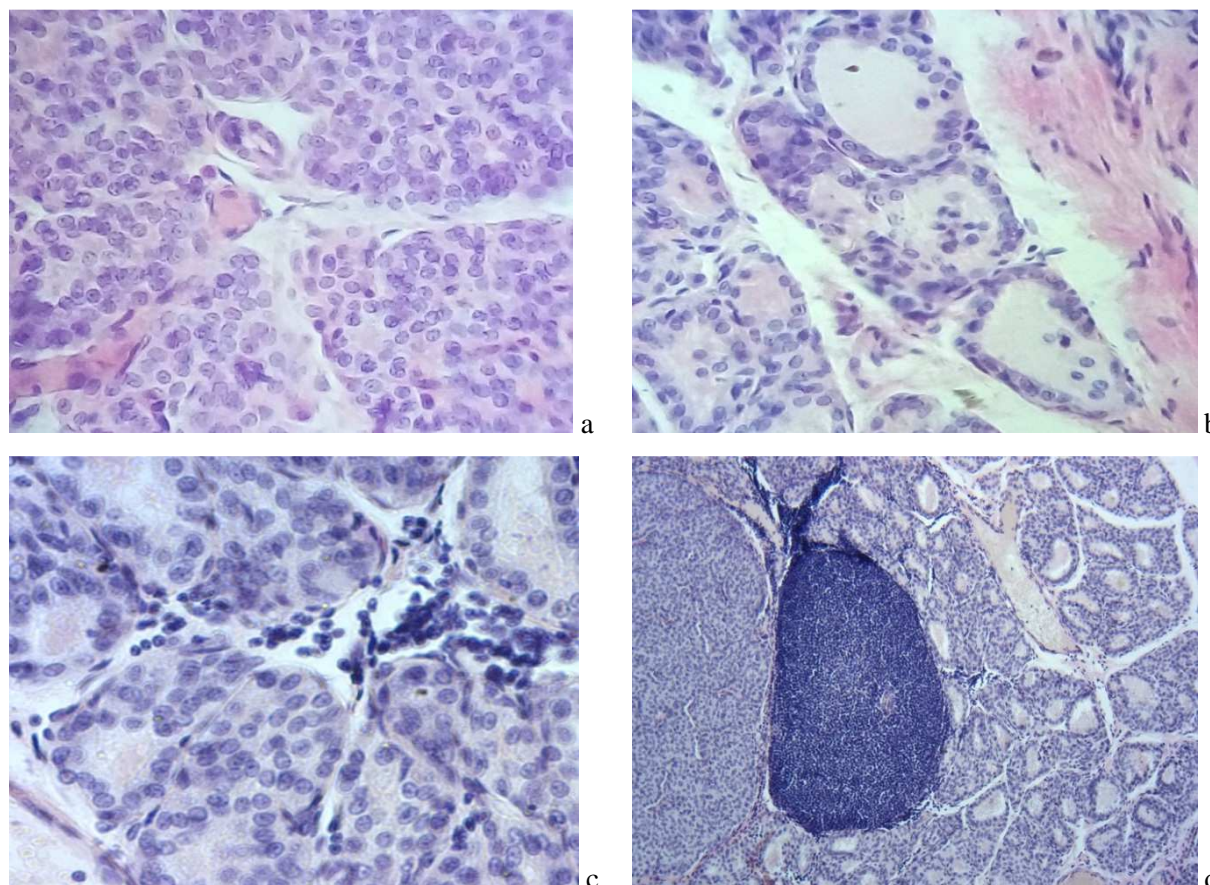


Fig. 1. Thyroid gland of suckling period rats on the 3rd day of life. a - intact group of animals; b - experimental group. $\times 600$. Lymphocytic infiltration in the thyroid gland of rats after prenatal exposure to staphylococcal toxoid. H&E stain. c - 7th day of life, $\times 600$. d - 21st day of life, $\times 150$.

In animals after intrauterine action of staphylococcal toxoid for 1-7 days, the average diameter of the nuclei is significantly reduced compared to the control group and is $0.25 \pm 0.01 \mu\text{m}$. There is a tendency to decrease the number of nucleoli in thyrocytes of large follicles and to increase in adenomers of medium and large diameters (tab. 2)

Table 2

Percentage of thyrocytes with different numbers of nucleoli in the follicles of suckling animals of control and experimental groups ($M \pm m$), $p < 0.05$

Group	Intact			Experimental		
	Numbers of nucleoli in thyrocytes			Numbers of nucleoli in thyrocytes		
Follicles	1	2	3 and more	1	2	3 and more
Large	22 \pm 2,03	30,7 \pm 1,05	49 \pm 1,03	19,7 \pm 0,04	32,7 \pm 3,02	47,7 \pm 3,04
Middle	22,7 \pm 4,05	33,7 \pm 3,01	44,3 \pm 2,03	29,7 \pm 2,05	23,7 \pm 3,04	46,7 \pm 2,01
Small	25 \pm 3,01	31,3 \pm 3,03	43,7 \pm 6,04	25,3 \pm 1,05	26,7 \pm 2,01	48 \pm 4,03

The decrease in the number of nucleoli in follicular thyrocytes indicates a decrease in the synthesis of ribosome subunits, and hence the gross synthesis of protein products. This is accompanied by a slowdown in the excretion of hormones into the bloodstream (absence of resorption vacuoles in the colloid). Stagnation of secretion in the follicle cavity can lead to stretching of its walls, causing signs of hypertrophy.

PAS reaction in the thyroid gland of antigen-premium animals show, that the colloid in large diameter follicles (++) has the most intense color. In medium and small adenomers, the reaction is less intense (+). The colloid of single follicles does not show PAS-positive properties. The cytoplasm of thyrocytes of follicles of different diameters is brightly colored over the entire area of the organ, but the intensity of the PAS reaction is more pronounced in prismatic and cubic thyrocytes, which correlates with manifestations of functional tension (synthesis and secretion of thyroid hormones).

Starting from the 7th day of life in antigen-premium animals in the subcapsular zone of the thyroid gland there are places of eviction of small and medium lymphocytes by diapedesis through the wall of the venules and their migration around the follicles (fig. 1c). Colloid-type adenomers in such locations on the 11th day already showed board vacuolation of the colloid (not a characteristic picture for the entire

parenchyma), cubic thyrocytes with 2-3 nucleoli. In the cells of the thyroid epithelium are often visible figures of mitosis. Most often, such diffuse clusters of lymphocytes were localized near the border of the thyroid gland with the parathyroid glands.

In the middle suckling period, the thyroid gland of animals of the intact and control groups was characterized by an increase in the number of colloid type follicles. Cuboidal thyrocytes was detected moderate board vacuolation of colloid, which indicates active hormone-producing activity.

In animals of the experimental group from the 7th to the 21st day of the postnatal period there was a more rapid rate of folliculogenesis and the development of connective tissue components of the stroma. Adenomers mostly of medium and large size with rapid board vacuolation of the colloid, lined with cubic thyroid epithelium. 2-4 nucleoli are present in the nuclei of thyrocytes. At the sites of lymphocyte migration into the connective tissue layers, near the venule wall, mast cells with metachromatic granules without signs of mass degranulation are detected. On the 14th day, a clearly formed, mostly single (lymphoid) nodule with lymphocytic cords (Fig. 1d) is visible, which consists mainly of small and medium lymphocytes, without a germinal center and a vaguely formed capsule. Desquamated cells of the thyroid epithelium are often visible in the colloid adenomers adjacent to the lymphoid nodule, while the shape of the follicles, the integrity of the epithelium and the basement membrane are not violated.

On the 21st day, in addition to diffuse lymphoid infiltration on the periphery of the thyroid lobes, the presence of a germinal center in the lymph node is noteworthy. The density of lymphocytes in the central zone of the nodule is lower than in the peripheral, among the lymphocytes of the central zone there are macrophages and plasma cells.

Thus, after the prenatal action of staphylococcal toxoid on the fetus, the rate of thyroid morphogenesis changes dramatically. This tendency of morphological changes of parenchymal organs is substantiated by a number of studies of other scientists [1, 3, 5, 7]. Thyroid folliculogenesis accelerates, but despite this, in the early dairy period on serial sections revealed morphological signs that indicate a decrease in the functional activity of the organ. Among them: an increase in the number of large and medium-sized follicles, a decrease in the height of the follicular epithelium, the lack of marginal vacuolation of the colloid. These data correlate with transformations at the cellular level, namely, there is a decrease in the number of nucleoli and a decrease in the concentration of RNA in the cytoplasm of thyrocytes, which indicates the suppression of synthetic processes occurring in the cell. With the advent of lymphocytic infiltration with the onset of the middle suckling period in the thyroid gland in large and medium follicles increases the height of the thyroid epithelium, the number of nucleoli in the nuclei of thyrocytes, increases regenerative-desquamation processes, begins resorption of colloid and active production of hormones in the blood by morphologically formed structures. Such abrupt changes in the thyroid gland of experimental animals are due to systemic prenatal antigenic influence of the body as a whole and has an adaptive-compensatory nature, both on the part of the immune system and the thyroid gland, because its hormones directly affect the body's development under endogenous and exogenous factors confirmed by other studies [2, 6, 8]. Thyroid transformation under prenatal antigenic influence on the fetus is an immunologically dependent dynamic process with sequential deployment and change of regular stages with the release of a polymorphnoplasic variant of the thyroid gland in suckling rats, and coincides with the concept of studies of various organs under antigenic conditions. [1, 3, 5, 7].

Conclusions

Intrauterine injection of staphylococcal toxoid led to the formation of a more pronounced structure of both parenchymal and stromal elements, but with morphological signs of functional immaturity and at the early stages of development with a morphological picture of signs of the thyroid gland's hypofunction. Structural and functional changes of the thyroid gland in the experimental group may be associated with the indirect influence of lymphoid cells on the morphogenesis of the organ, which is the purpose of further study. The obtained data are important for pediatricians, endocrinologists, immunologists in explaining certain etiopathogenetic aspects of autoimmune thyroiditis in children.

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Реферати

МОРФОГЕНЕЗ ЩИТОПОДІБНОЇ ЗАЛОЗИ ЩУРІВ МОЛОЧНОГО ПЕРІОДУ ПІСЛЯ ПРЕНАТАЛЬНОЇ ДІЇ СТАФІЛОКОКОВОГО АНАТОКСИНУ

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Одержано результати щодо темпів морфогенезу щитоподібної залози після внутрішньоплідної антигенної дії стафілококкового анатоксину. Пренатальне введення стафілококкового анатоксину призвело до формування більш вираженого структуроутворення елементів паренхіми і стромы, але вони мали ознаки функціональної незрілості, що призвело до наявності морфологічної картини гіпотиреозу вже після народження (щери 1-7 доби постнатального онтогенезу). З початком середнього молочного періоду (7-21 доба життя) з'являється лімфоцитарна інфільтрація в окремих ділянках щитоподібної залози, відбувається пребудова синтетичного апарату та процесу резорбції колоїду, тобто починається функціональне «дозрівання» вже морфологічно сформованих структур. Такі скачкоподібні зміни в щитоподібній залозі експериментальних тварин обумовлені системним пренатальним антигенним навантаженням організму в цілому та носить пристосувально-компенсаторний характер.

Ключові слова: щитоподібна залоза, антиген, стафілококковий анатоксин, морфогенез, експеримент.

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МОРФОГЕНЕЗ ЩИТОВИДНОЇ ЖЕЛЕЗЫ КРЫС МОЛОЧНОГО ПЕРИОДА ПОСЛЕ ПРЕНАТАЛЬНОГО ДЕЙСТВИЯ СТАФИЛОКОККОВОГО АНАТОКСИНА

Федосеева О.В.

Получены результаты о темпах морфогенеза щитовидной железы после внутриплодного антигенного воздействия стафилококкового анатоксина. Пренатальное введение стафилококкового анатоксина привело к формированию более выраженного структурообразования элементов паренхимы и стромы, которые имели признаки функциональной незрелости, что привело к наличию морфологической картины гипотиреоза уже после рождения (крысы 1-7 суток постнатального онтогенеза). С началом среднего молочного периода (7-21 сутки жизни) появляется лимфоцитарная инфильтрация в отдельных участках щитовидной железы, происходит перестройка синтетического аппарата и процесса резорбции коллоида, то есть начинается функциональное «созревание» уже морфологически сформированных структур. Такие скачкообразные изменения в щитовидной железе экспериментальных животных обусловлены системным пренатальным антигенным воздействием на организм в целом, и носит приспособительные-компенсаторный характер.

Ключевые слова: щитовидная железа, антиген, стафилококковий анатоксин, морфогенез, експеримент.

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ULTRASTRUCTURAL ORGANIZATION FEATURES OF PERIODONTAL TISSUES AFTER TWELVE WEEKS OF OPIOID INFLUENCE

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In the experiment on white rats, ultrastructural studies of periodontal tissues were carried out against the background of twelve-weeks opioid action. Animals were subjected to intramuscular injections of an opioid analgesic with a gradual increase in the mean single dose every two weeks. The initial dose was 0.212 mg/kg, and within 11–12 weeks the dose was increased up to 0.3 mg/kg. The results of submicroscopic studies have revealed the progression of chronic sclerosing inflammation in the periodontium and the development of regenerative-plastic deficiency of epitheliocytes, endothelial cells and the periodontium structural components, which was caused by a long-term opioid action.

Key words: periodontium, rat, opioid, ultrastructure.

The work is a fragment of the research project “Morpho-functional features of organs in the pre – and postnatal periods of ontogenesis, under the influence of opioids, food supplements, reconstructive surgery and obesity”, state registration No. 0120U002129.

Periodontal diseases of a dystrophic-inflammatory nature are quite widespread in practically most age groups with more than 75% of the population affected worldwide, which is an important socio-medical problem [1, 3, 6, 12, 13]. In particular, a number of researchers consider generalized periodontitis a polyetiological disease with various mechanisms of pathogenesis [5, 6, 13]. In numerous disorders of metabolism that cause the development of morphological manifestations in periodontal tissues, complications occurring under the influence of narcotic intoxication play a significant role [4, 8]. It should be noted that the pathology of organs and tissues in the oral cavity of opioid-dependent persons has not been sufficiently