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THE FOLLICLEGENESIS IN THE THYROID GLAND IN THE POSTNATAL PERIOD OF ONTOGENESIS UNDER PRENATAL ANTIGENIC INFLUENCE

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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. The paper was aimed at the study of the folliclegenesis of the thyroid gland in postnatal period at norm and after prenatal influence of staphylococcal toxoid. In the experimental research as a material were thyroid glands of Wistar rats aged 1 to 60 days of postnatal development (162 animals), about 6 animals in each group. Three animals groups were studied on 1, 3, 7, 11, 14, 21, 30, 45, 60 days after bith. I gr. - intact animals (norm); II gr. - control, animals which were injected intrauterine 0.9% NaCl solution; III - experimental animals injected with staphylococcal toxoid liquid purified adsorbed (10-14 units of binding in 1 ml, diluted 10 portions) by operation intrauterinely on the 18th day of dated pregnancy. Histological sections 3-5 μm thick were stained by hematoxylin and eosin, histochemicaly by alcian blue and azan staining. Immunohistochemical study was performed according to the protocol recommended for a particular antibody of the manufacturer. Used ki-67 (Ki-67), TTF-1 (8G7G3/1), Fox-1 (A-12) monoclonal antibodies by Santa Cruz Biotechnology, Inc. 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. For processing of statistical material was used the standard software package Microsoft Office Excel and Statistica 10.0.

The results were obtained about morphogenesis of rat's thyroid after intrauterine antigenic action of staphylococcal toxoid. Morphofunctional homeostasis and stromal-parenchymal proportional relationship to thyroid gland closely associated with the activity of immune cells, including special role of lymphocytes, macrophages, and mast cells. 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 after birth. During the sucking period, the simultaneous presence of intra-, extrafollicular, septal and intramural types of folliculogesis is determined, which is a local reaction to systemic antigenic irritation with activation of compensatory-adaptive reactive folliclegenesis. The revealed changes in the process of folliclegenesis, accompanied by venous plethora, the formation of intraorgan diffuse lymphoid tissue and nodules, desquamation of the follicular epithelium, redistribution of the follicle diameter is a reaction to the systemic antigenic effect on the body during the critical period of prenatal development and normalizes by 45 days.

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

Introduction. Thyroid gland is an important endocrine organ, the action of hormones which variously aimed at all the metabolic processes function of many organs and tissues, including fetal development, the growth and differentiation of tissues [1, p.e0221939; 2, p.215-223] .

The mechanisms by which infection may, theoretically, induce an autoimmune response are many, and this makes infections an attractive hypothesis for disease initiation. Yet here we run into some difficulties because the literature is weak and polluted. Immunologically speaking, the body has a number of ways to recognize external danger, and this results in a fierce immune attack on the culprits. These reactions, however, may sometimes go astray and damage not only the external cause of the danger, such as bacteria, but also the endogenous standby—oneself [3, p.298-306]. The immune system, which evolved to defend us from invading foreign proteins, normally tolerates (i.e. does not develop recognizable responses to self-antigens [4, p.111-118]. The level of this control is variable. However, antibodies to thyroid antigens exist in up to 20% of adult women, and their presence must be considered effectively normal. The development of tolerance is closely associated with the restriction of TCRs to recognizing an antigen only when presented by an HLA molecule. The process, which for T cells occurs in the fetal thymus, leads to elimination of some T cells, and retention of others with TCRs having desirable features. Self-antigens are believed to be presented on HLA molecules to T cells developing in the thymus [3, p.298-306; 5, p.151-157]. This implies that antigen must be in the thymus or in the circulation for tolerance to develop and indeed we now know that specialized cells in the thymus can express a panoply of autoantigens during development [6, p.214-219]. T cells bearing autoreative TCRs are largely inactivated or destroyed. T cells which have the capacity to react with foreign antigens presented by self MHC molecules are allowed and retained. This system is imperfect however and some T cells which react with MHC molecules plus self-antigen are not deleted, which is the fundamental explanation for autoimmunity. The ontogeny and functions of T and B cells have been identified in a variety of ways, including morphologic and functional criteria, and by antibodies

identifying surface proteins which correlate to a varying extent with specific functions [7, p.320-328; 8, p.58-69].

Thus, the study of the prenatal effect of a foreign protein (staphylococcal toxoid) on the ways of folliclegenesis of the thyroid gland after birth is a topical direction.

Purpose. The paper was aimed at the study of the folliclegenesis of the thyroid gland in postnatal period at norm and after prenatal influence of staphylococcal toxoid.

Material and Methods. In the experimental research as a material were thyroid glands of Wistar rats aged 1 to 60 days of postnatal development (162 animals), about 6 animals in each group. Three animals groups were studied on 1, 3, 7, 11, 14, 21, 30, 45, 60 days after bith. I gr. - intact animals (norm); II gr. - control, animals which were injected intrauterine 0.9% NaCl solution; III - experimental animals injected with staphylococcal toxoid liquid purified adsorbed (10-14 units of binding in 1 ml, diluted 10 portions) by operation intrauterinely on the 18th day of dated pregnancy. Injections of antigen or 0.9% NaCl solution for fetus were performed surgically during laparotomy, by intrauterine, transdermal subcutaneous at a dose of 0.05 ml to each fetus. The distribution of the material is presented in table 1.

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 μm thick were stained by hematoxylin and eosin, histochemicaly by alcian blue and azan staining.

Table 1

Distribution of research material

Age groups of the dairy period	Day of a life	Intact group	Control group	Experimental group
Newborns (early dairy)	1	6	6	6
	3	6	6	6

	7	6	6	6
Sucking (average dairy)	11	6	6	6
	14	6	6	6
	21	6	6	6
Infantile (late dairy)	30	6	6	6
	45	6	6	6
Premarital (juvenile)	60	6	6	6
Total		**54**	**54**	**54**

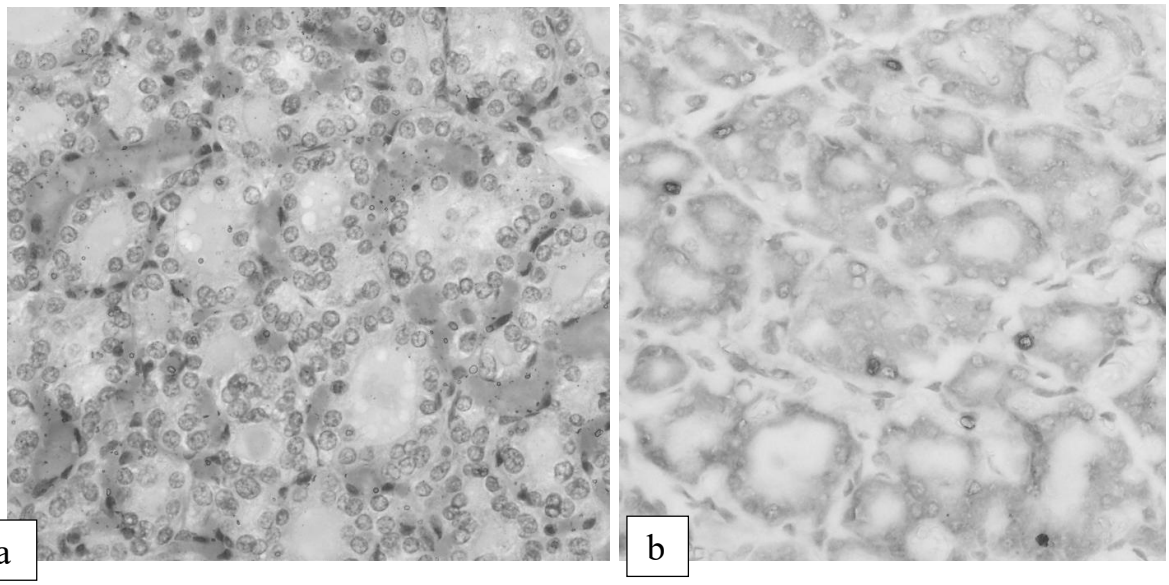
Immunohistochemical study was performed according to the protocol recommended for a particular antibody of the manufacturer and was performed to assess the proliferative activity in the parenchyma of the thyroid gland and identification of groups of lymphocytes in lymphoid tissue. Used a proliferation marker ki-67 (Ki-67), TTF-1 (8G7G3/1), Fox-1 (A-12) monoclonal antibodies by Santa Cruz Biotechnology, Inc. using the method of indirect staining with immunoperoxidase, using conjugated HRP murine IgG-binding proteins, m-IgGκ BP-HRP, followed by incubation in peroxidase substrates and a mixture of chromogen DAB-3-diaminobenzidine tetrachloride and repainting of the nuclei with Mayer hematoxylin, dehydration, enlightenment and cover in balm.

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 $p \leq 0,05$. For processing of statistical material was used the standard software package Microsoft Office Excel and Statistica 10.0.

Results and Discussion

Newborns (early dairy) period (1-7 days). Microscopic study of serial sections thyroid experimental animals aged 1-7 days, notes recalibration of arteries, capillaries expansion, an increase in thyroid stromal component between large follicles. There is a prevalence of large diameter follicles throughout the volume of the body. There are single and grouped cystic

follicles. The number of follicles small diameter was significantly reduced compared with the control group. In the stroma, changes are manifested by hyperemia and stasis in the blood vessels (pic. 1, a), along the periphery, mainly from the subcapsular connective tissue, thin layers of the connective tissue of the organ are infiltrated by lymphocytes. The expansion and abundant filling of the interfollicular vessels of the thyroid gland with blood is a condition in which it is very convenient to observe and study all the features of the processes of its proliferation. This especially applies to the moment of budding of cells from the follicle wall, location, shape of interfollicular islets, their relationship with blood vessels and connective tissue. In experimental group the number of thyrocytes with positive nuclear expression of Ki-67 (pic. 1, b) increased for the 7th day by 3,63 times (from 1,6±043 to 5,8±0,62) and in comparison with the control group by 1,9 (3,1±0,34). During this period, folliclegenesis takes place by dividing thyrocytes inside the follicular wall. There is also a proliferative activity of thyrocytes in interfollicular islets of a colloid-free type of secretion united by a common basement membrane. During the neonatal period in animals of the experimental group, folliclegenesis proceeds by intramural follicular proliferation without the formation of extra points and intrafollicular growth. Mitotic activity during this period increases with peak values on the third day and an insignificant decrease on the seventh day, which correlates with the values of Fox-1 expression, while the cytoplasmic expression of Fox-1 increases uniformly by day 7, and nuclear expression fully corresponds to the tendency of mitotic division. Expression of TTF1 is directly proportional to Fox-1 nuclear expression, which proves the link between these two factors.



Pic. 1. Thyroid gland of animals in early dairy period from experimental group

a – noteworthy is the expansion and plethora of interfollicular venules, hemostasis. Stain by hematoxilin & eosin, x 600. **b** - immunohistochemical reaction with Ki-67 in nucleases of proliferative thyrocytes. IHC staining with ki-67 with repainting by Mayer hematoxylin. x 600.

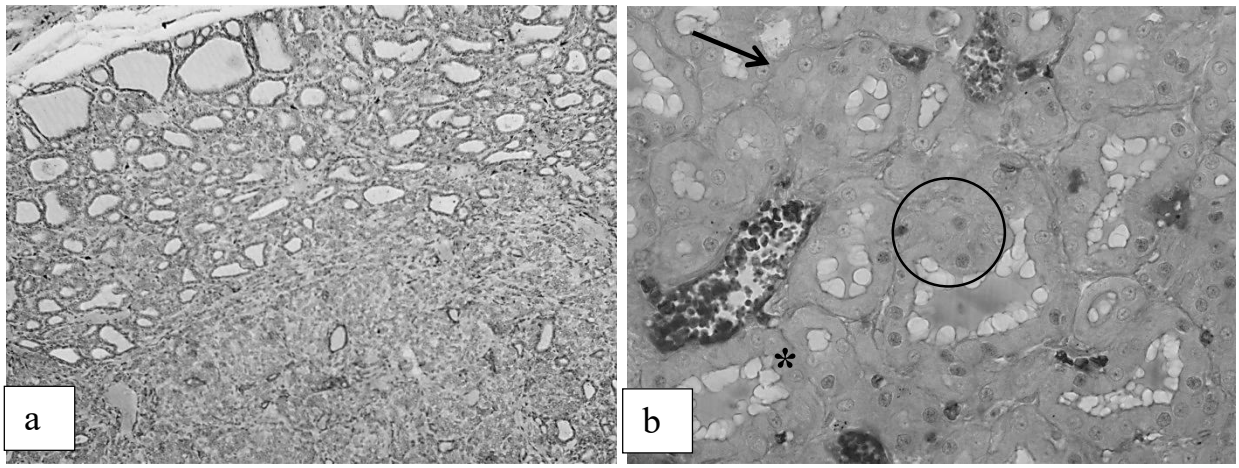
Sucking(average dairy) period. In the thyroid gland of rats, there is a clear division of the parenchyma into a peripheral and central zone (pic. 2, a). In the peripheral zone, the follicles are large, C cells are absent, and in the central zone there are small follicles with a large number of cells, this is a phenomenon of stratification. In this period folliclegenesis is most intensive in thyroid of experimental animals. Folliclegenesis of the peripheral zone is carried out by fragmentation of large follicles into several small, sprouting cords of connective tissue. In parallel with intense parenchymal changes, significant changes in the stroma occur, which undoubtedly affects the thyroid epithelial proliferative activity. So, during this period, lymphoid accumulations are formed in the form of a nodule or diffuse lymphoid tissue in the peripheral part of the thyroid lobes; there is also an increase in the number of mast cells compared to the control group

and the proliferation of connective tissue with coarsening of collagen. In some follicles of the peripheral part of the lobes of the thyroid gland, desquamation of the epithelium into the follicle is noted. This tendency is especially noted in places of lymphoid infiltration. All this is undoubtedly stimulating factors of intensive folliclegenesis in response to prenatal immune stimulation by staphylococcal toxoid.

At the same time, in the thyroid gland of experimental rats of this age period, visualized two types of folliclegenesis, wich are simultaneously observed: central and peripheral, which are characteristic of the corresponding zones. In the process of central folliclegenesis, the following stages can be distinguished: 1) first, an epithelial buds appears in the follicle cavity, which necessarily has a connective tissue base in the center, that is, an epithelial buds is a connective tissue cord that introduces the follicle covered with a layer of follicular thyrocytes; 2) at the next stage, the rounded follicle is divided by ingrowing bands of connective tissue into two flattened follicles; 3) flattened follicles are also fragmented into several small follicles by sprouting cords of connective tissue as vectors of forces. In the process of peripheral folliclegenesis, another way of follicle fragmentation is observed: 1) several small connective tissue cords grow into the follicle cavity at an angle to each other; 2) then connecting with apical parts, they separate small follicles from a large one. Sometimes follicles can be observed, the epithelial wall of which consists of many small follicles.In relation tissue components showed a reduction in the specific area of thyroid epithelium. This is because the height of follicular epithelial cells become less prevalent and cubic shape thyroid flat, cylindrical cells are rare, mainly in small follicles (pic. 2, b).

Found relative growth rate colloid area due to the greater number of large follicles, increasing the average diameter adenoma containing dense, thick colloid. In the oral follicles are desquamated cells. The colloid

vacillation not found. From the 7th day, points of intra- and extrafollicular growth begin to appear. At the same time, proliferative activity increases rapidly. The expression of Ki-67 is observed both in cell growth clusters and in the monolayer follicular epithelium and increases by 11th day with maximum values of 42,08±1,3, which is 4,2 times more in comparison with the first day of postnatal life.



Pic. 2. Thyroid gland of animals in average dairy period from experimental group

a – 11th day after birth. Visualization of the peripheral and central part of the thyroid gland. IHC staining with TTF-1 with repainting by Mayer hematoxylin, x300. **b** – 14th day after birth. Three types of folliclegenesis: circle – intrafollicle growth; arrow – extrafollicle proliferation; * - follicle division by septation. Histochemical staining by alcian blue and azan. x 600.

The proliferation vector is aimed at thyroid specification, which is expressed corresponding to the expression of immunohistochemical markers Fox-1 and TTF-1. By the 14th day, the processes of intra-, extrafollicular growth and proliferation by dividing the follicle (septation) are visualized. Thus, 14 days can be considered the peak period of adaptogenic and compensatory proliferation in response to prenatal immune stimulation. In the period from 14 to 21 days, the proliferative

activity in the parenchyma of the thyroid gland of experimental animals is kept practically at the same level, but decreases compared to the previous age on 1,8 by the number of epithelial clusters of the follicle wall.

Infantile (late dairy) period. On the 30th day, the number of thyroid clusters decreases compared to the previous period by 1,09 and is 14,33±0,53 respectively, but the number of cells with cytoplasmic Fox-1^{+} expression increases, which indicates the maturation and specification of the synthetic apparatus of secretory endocrine cells and the formation of secretory activity. Thus intracellular proliferation of thyrocyte's organelles occurs during this period. By the 45th day, the formation of extra- and intrafollicular clusters sharply decreases and folliclegenesis decreases, however, intramural proliferation of thyrocytes and intracellular formation of a specific synthetic apparatus remain, as evidenced by the presence of positive expression of Ki-67, TTF-1 and Fox-1, while the number of cells with positive expression Ki-67 in the follicle wall practically does not differ from such positive expression of TTF-1.

Conclusions.

1. In response to the prenatal antigenic load, the body reacts to the immune system, which also acts on the thyroid gland. Accordingly, the experimental group revealed aberrant morphofunctional changes that affect the maturation and formation of both the parenchyma and the stroma of the thyroid gland.

The types of folliclegenesis have been established and described. During the sucking period, the simultaneous presence of intra-, extrafollicular, septal and intramural types of folliclegesis is determined, which is a local reaction to systemic antigenic irritation with activation of compensatory-adaptive reactive folliclegenesis.

2. The revealed changes in the process of folliclegenesis, accompanied by venous plethora, the formation of intraorgan diffuse

lymphoid tissue and nodules, desquamation of the follicular epithelium, redistribution of the follicle diameter is a reaction to the systemic antigenic effect on the body during the critical period of prenatal development and normalizes by 45 days.

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