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AUTOIMPLANTATION OF THYMUS IN SURGICAL CORRECTION OF CONGENITAL HEART DEFECTS

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ABSTRACT: *The research presents the results of a comprehensive immunomorphological study in thymus autoimplantation. Using morphological and immunological research methods, was assessed the state of the epithelial reticular stroma of the thymus in experimental Chinchilla rabbits. The clinical and immunological effects of the influence of autoimplantation on the stages of experimental research have been established.*

Keywords: thymus, autoimplantation, rabbits, immunology

Introduction

It is known that most congenital heart defects(CHD) in young children is accompanied by a change in systemic hemodynamics with impaired blood flow, both in the cavities of the heart and in the large and small circles of blood circulation. CHD are among the most frequent developmental anomalies in children [17]. According to research results, children with CHD are often susceptible to various diseases due to their existing immune imbalance [3, 17, 9,8,11].

Most often, hypoxia occurs with the development of heart defects. One of the most frequent pathogenetic processes underlying the disorder of the structure and function of the thymus is hypoxia. Hypoxia as a stress factor is still an object of close study by specialists in various fields, since it develops metabolic and structural changes at the cellular, tissue and organ levels [10].

The urgency of the problem is associated not only with the prevalence, but also with the growth trend of severe combined CHD with frequent unfavorable outcomes, especially in the first months of life of children [2]. Currently, the bulk of research is devoted to assessing the postoperative state of the immune system of children with CHD after thymectomy [2, 5]. However, the question of the morpho-functional state of the thymus as the primary organ of immunity and its provision of the developing organism*T*- lymphocytes under conditions of circulatory hypoxia remains unresolved today.

As a rule, in the postoperative period, the main task of the cardiologist and pediatrician is aimed at adapting the child's cardiovascular system. The assessment of the immune system of this category of patients in dynamics is not carried out. It is known that the fate of the immune system in childhood is determined by the state of the primary organ of immunity - the thymus. Violation of its

structure and function in various diseases of an infectious and non-infectious nature can be the cause of the development of accidental involution of the organ, determining the defective state of the immune system as a whole [2,5]. We have studiedmorphofunctional features of the thymus after autoimplantation.

Purpose of the study: the study of the morphofunctional features of the state of the thymus tissue after autoimplantation of the thymus during surgical correction of congenital heart disease.

Materials and methods: Thymus material examined45 Chinchilla rabbits of both sexes, in at the age of 3 months of life with a mass of 1790 - 2495 g., withheld in standard vivarium conditions - at a temperature of 22-24 ° C, a relative humidity of 50-60% and a light regime (12 hours of darkness and light). Highlighted 2 groups15 heads each: one- group - an experimental group who underwent total thymectomy followed by auto-implantation of the thymus; Group 2 - laboratory animals that underwent only total thymectomy. The comparison group was left with healthy rabbits (intact animals) of the corresponding age, which were kept in standard vivarium conditions.Peripheral blood (*n*= 45) was taken from the auricular vein (1.5 ml.) rabbits taken before the experiment, and on the 1^{st}, $7^{th}$and 9^{th}weeks after the experiment. Blood for research was taken into medical test tubes by puncturing the marginal auricular vein of rabbits with an injection needle. The number of samples is 15 from each grouprabbits. Histological, immunohistochemical, microscopic examinations were carried out.

Histological examination. The thymus was fixed in 12% neutral formalin in phosphate buffer (*pH* 7.2) and embedded in "Histomix" paraffin. Sections 4-5 μm thick were stained with hematoxylin and eosin to assess the overall morphological picture. A morphological study of the thymus was carried out in the periods before and after auto-implantation at the Central Scientific Research Laboratory of BSMI.

Morphological and biochemical analyzes blood tests were carried out in the laboratory of LLC "Standard Diagnostika" (Bukhara).

Immunological studies were carried out at the Institute of Immunology and Human Genomics of the Academy of Sciences of the Republic of Uzbekistan (Tashkent). *T*-lymphocytes were determined by ELISA: young thymocytes (CD3 +, CD4 +, CD8 +) - involved in antigen-independent differentiation*T*-cells, natural killer cells (EK-CD16 +), lymphocytes of early (CD25 +) and late activation (CD95+).

The primary adaptation of rabbits was carried out for an equalizing period of 14 days at the Central Scientific Research Laboratory of BSMI. Keeping laboratory animals, feeding and caring for them, selection of animals, cleaning and disinfection of the vivarium premises were carried out in accordance with the approved recommendations [11].

Experimental studies were carried out in two stages: the first stage - modeling thymectomy in groups I-IIfrom compliance with all the rules of asepsis and antiseptics. Under general anesthesia: 5% Calypsol solution was injected intramuscularly at the rate of 6-8 mg / kg. In experimental rabbits of the first group, after extraction of the thymus, auto-implantation of 1/3 of the organ on the pericardium in the projection of the right atrium was performed. The thymus was fixed with 5.0 Vicryl suture. Hemostasis, the wound was sutured in layers and an aseptic bandage was applied after the wound was treated with 10% Betadine solution. The rest of the thymus was fixed with 12% formalin solution for histochemical studies.

The second stage - the animals were slaughtered by a humane method and the morphological assessment of changes in the autoimplant (thymus) was carried out: pathomorphological changes in

the thymus during auto-implantation, the total pool of *T*-cells in the blood, clinical blood analysis and statistical methods of research. When working with laboratory animals, all biological safety rules and ethical principles of working with laboratory animals were observed [11].

Statistical processing was carried out using generally accepted methods of variation statistics (Microsoft Excel office program, version 2019 on the Microsoft Windows 10 platform). Continuous independent variables with normal parametric distribution are used. To compile an idea of the sample as a whole, the principles of descriptive statistics and the indicators mean (M) and discrete data presented as a proportion (%) were used. For a comparative assessment of indicators, M and standard deviation (m) were indicated. The level of significance of the indicator of the reliability of differences was considered * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$.

Discussion: The age characteristics of the peripheral blood parameters were taken into account in the course of the study.Before the experiment, indicators of a general blood test (leukocytes, erythrocytes, hematocrit, hemoglobin, ESR) in experimental rabbits of all groups were at the level of control values (Fig. 1).

On the 7thday (at the end of the 1st week) of the experiment (autoimplantation) in the experimental rabbits of the 1st group it was noted:

-leukocytosis up to 11.8 ± 2.45 x 109 / l versus control indicators (6.3 ± 0.79 x 109 / l) ($p < 0.05$);

- erythrocytopenia up to 4.3 ± 0.55 g / l versus control values (6.4 ± 0.76 g / l) ($p < 0.05$);

- acceleration of ESR to 11.4 ± 3.44 mm / h in relation to the indicators of the control group (3.6 ± 0.55 mm / h) ($p < 0.05$).

The data obtained were reliable in relation to the indicators of the control group and were assessed as the body's response to the stress factor (surgical correction with autoimplantation of the thymus).

In the 7thweek of the experimentin experimental rabbits of the 1st group, it was noted:

-the tendency to normalize the level of leukocytes up to 6.5 ± 0.56 x 109 / l to control values (7.5 ± 0.57 x 109 / l) of rabbits of the corresponding age;

-normalization of the level of erythrocytes to 5.0 ± 0.55 g / l versus the indicators of the control group (6.1 ± 0.48 g / l);

-normalization of ESR to 6.7 ± 1.82 mm / h in relation to control indicators (5.8 ± 0.43 mm / h).

On the 9thweek of the experimentin experimental rabbits of the 1st group, it was noted:

-normalization of the level of leukocytes up to 5.4 ± 0.74 x 109 / l to the level of control values (5.6 ± 0.79 x 109 / l) of rabbits of the corresponding age; normalization of the level of erythrocytes to 5.5 ± 0.64 g / l versus control indicators (6.4 ± 0.76 g / l); decrease in ESR to 3.1 ± 0.79 mm / h in relation to control indicators (4.9 ± 1.62 mm / h) (Table 1 and Fig. 1).

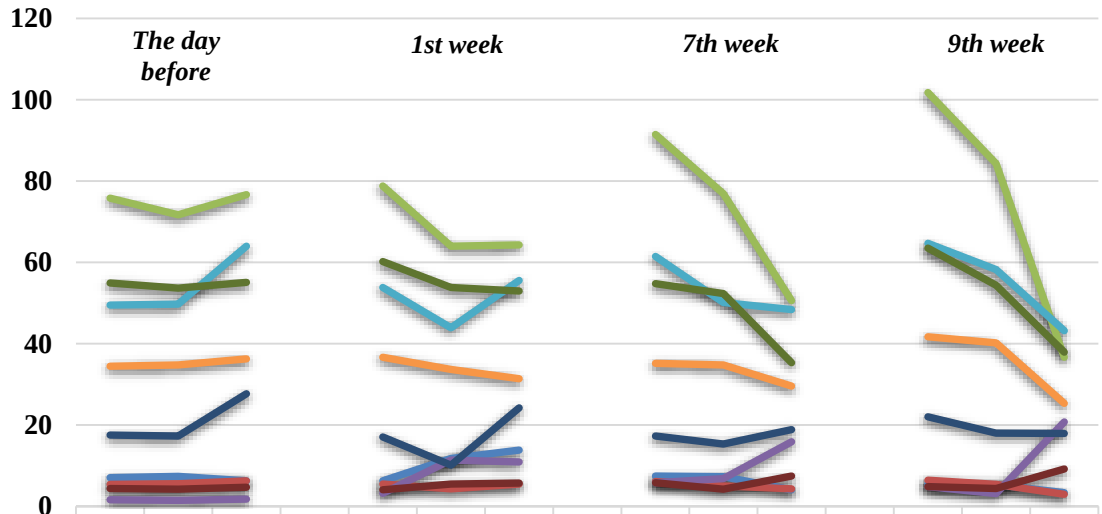
Table 1

Indicators of a general blood test in experimental rabbits

Before experiment	Leukocytes 109 / l	Erythrocytes 1012 / l	Hemoglobin g / l	ESR mm / h
Control group	*7.1 ± 0.87*	*5.4 ± 0.79*	*75.8 ± 6.01*	*1.6 ± 0.33*
1stgroup	*7.4 ± 0.59*	*5.6 ± 0.91*	*71.7 ± 6.76*	*1.5 ± 0.33*
2ndgroup	*6.3 ± 0.76*	*6.3 ± 0.59*	*76.7 ± 7.14*	*1.8 ± 0.43*

1st week	Leukocytes 109 / l	Erythrocytes 1012 / l	Hemoglobin g / l	ESR mm / h
Control group	6.3 ± 0.76	5.5 ± 0.72	78.8 ± 4.01	3.2 ± 0.32
1st group	11.8 ± 2.45*	4.3 ± 0.55	64 ± 4.77 *	11.4 ± 3.44 *
2nd group	13.2 ± 2.71*	5.5 ± 0.94	61.6 ± 4.4 *	10.9 ± 2.81 *
7th week	**Leukocytes 109 / l**	**Erythrocytes 1012 / l**	**Hemoglobin g / l**	**ESR mm / h**
Control group	7.5 ± 0.57	6.1 ± 0.48	91.5 ± 3.52	5.8 ± 0.43
1st group	6.5 ± 0.56	5.0 ± 0.55	76.2 ± 2.48 *	6.7 ± 1.82
2nd group	4.1 ± 0.79 *	4.3 ± 0.71 *	50.6 ± 5.96 **	16.1 ± 3.43 *
9th week	**Leukocytes 109 / l**	**Erythrocytes 1012 / l**	**Hemoglobin g / l**	**ESR mm / h**
The control	5.6 ± 0.79	6.4 ± 0.76	101.8 ± 5.32	4.9 ± 1.62
1st group	5.4 ± 0.74	5.5 ± 0.64	84.3 ± 4.1 *	3.1 ± 0.79
2nd group	3.4 ± 0.6 *	3.0 ± 0.38 *	42.5 ± 1.64 **	20.4 ± 5.28 *

*Note: * indicators are reliable in relation to control:*
** -p <0.05; ** p <0.01*



	CRTL	1st	2nd	CRTL	1st	2nd	CRTL	1st	2nd	CRTL	1st	2nd
WBC 109/L	7,1	7,4	6,3	6,3	11,8	13,8	7,5	7,3	4,1	5,6	5,3	3,4
RBC 1012/L	5,4	5,6	6,3	5,5	4,3	5,5	6,1	5	4,3	6,4	5,4	3
Hb g/L	75,8	71,7	76,7	78,8	64	64,3	91,5	76,9	50,6	101,8	84,3	36,7
ESR mm/h	1,6	1,5	1,8	3,2	11,4	10,9	5,8	6,8	15,9	4,9	3,1	20,8
Protein serum g/L	49,5	49,7	64	53,8	43,9	55,6	61,5	50,1	48,4	64,7	58,2	43,2
Albumine g/L	34,5	34,8	36,3	36,7	33,7	31,4	35,2	34,8	29,6	41,7	40,2	25,3
Globuline g/L	17,5	17,3	27,7	17,1	10,1	24,2	17,3	15,3	18,9	22	18	17,9
Urea mmol/L	4,4	4,2	4,7	4,1	5,5	5,7	5,8	4,2	7,5	4,9	4,4	9,2
Creatinine µmol/L	54,9	53,7	55,1	60,2	53,8	53	54,8	52,3	35,3	63,5	54,4	37,9

Fig. 1. Blood parameters in experimental rabbits

During the first 9 weeks of observation after thymectomy, experimental rabbits of the 2nd group develop severe anemia, erythrocytopenia, leukopenia, accelerated ESR up to 20 mm / h, hypoproteinemia, hypoalbuminemia, uremia (Fig. 1).

The results of quantitative determination of *T*-lymphocytes in all groups on the day before the experiment were at the level of age values (Fig. 2). Over the course of 7 weeks of the experiment, a decrease in the number of CD3 + *T*-lymphocytes to 32.0 ± 5.35% and 15.2 ± 3.11% in the 1st and 2nd groups, respectively, in relation to the indicators of the control group 67.6 ± 7.09% ($p < 0.05$). At the same time, a deeper weakening of thymopoiesis was noted in rabbits of the 2nd group of the experiment. At the same time, the number of CD3 + lymphocytes in rabbits of the 1st group are 2.1 times higher than in the rabbits of the 2nd group ($p < 0.05$), which confirms the clinical and immunological effectiveness of autoimplantation of the thymus (Fig. 3.4).

On the 9th week of the experiment, a decrease in the number of CD3 +, CD4 +, CD8 + lymphocytes were found in rabbits of the 1st and 2nd groups in relation to the control. At the same time, a weak tendency towards a decrease in the indicated groups of lymphocytes was established in rabbits of the 1st group than in rabbits of the 2nd group. Thus, the number of CD3 + *T* cells in the blood of rabbits of group Iwere 2.98 times higher (up to 38.8 ± 3.17%) than in rabbits of group II (13 ± 2.97%) ($p < 0.01$). This trend continued for CD4 + CD8 + lymphocytes (Fig. 5).

Natural killers do not depend on the thymus for their development. They express on their surface receptors for interferon-γ and interleukin-2 (IL 2). Functionally, they are killer cytotoxic cells, but NK lacks antigen-recognizing receptors, which are necessarily present on killer *T* cells. Natural killer cells on the target cell are induced by IgG antibodies specific to the membrane antigens of the target cell. Initially, antibodies bind to the antigen on the cell, and then using the Fc receptor to IgG (FcγRIII) NK attaches to this AB-AG target cell complex. The function of NK cells in the body is to protect against the development of tumors, viruses, etc. Their main markers are CD16 and CD56. (FcγRIII according to CD nomenclature is CD16) [1].

In the experiment, during 9-weeks of observation in rabbits of the 2nd group, an increase in the relative number of CD16 + lymphocytes were found to 27.4 ± 4.88% versus the 1st group - 20.6 ± 6.77%.

Along with other proteins, soluble differentiation molecules produced by cells of the immune system are involved in the regulation of immunity [4,12,13,16].

Thus, soluble forms of CD25 molecules (sCD25+) are present in the blood. In membrane form, they are present on activated lymphocytes, including regulatory *T*-cells. The CD25 molecule (IL-2R) is the alpha chain of the interleukin 2 receptor. When lymphocytes are activated, the membrane expression of CD25 increases. Soluble CD25 molecules are produced by activated lymphocytes. The serum level of sCD25 serves as a marker of their activation in various pathological conditions [16].

By interacting with interleukin 2, soluble sCD25 molecules feedback inhibits immune responses, limiting an overdeveloped immune response when it is overactive. In this regard, sCD25 molecules are considered as a suppression factor for the immune response.

Studies have shown an increase in the level of CD25 + cells in rabbits of the 1st group of the experiment, which confirms the activation of lymphocytes in response to autoimplantation (Fig. 2).

The CD95 (Fas) molecule modulates cell apoptosis and also serves as a marker for lymphocyte activation. CD95 is a type I membrane protein with a molecular weight of 43 kDa, which is a member of the tumor necrosis factor receptor family and contains a death domain. This molecule is expressed in humans on cortical thymocytes, activated *T* and *B* lymphocytes, monocytes and neutrophils. In addition, outside the immune system, CD95 is detected on various types of normal human cells [14].On the 9thweek of observation,in the experimental rabbits of the 2ndgroup, after total thymectomy, was established the increaseof the level of CD95-lymphocytes, which indicates the activation of lymphocytes carrying apoptosis antigens on the surface of cells.

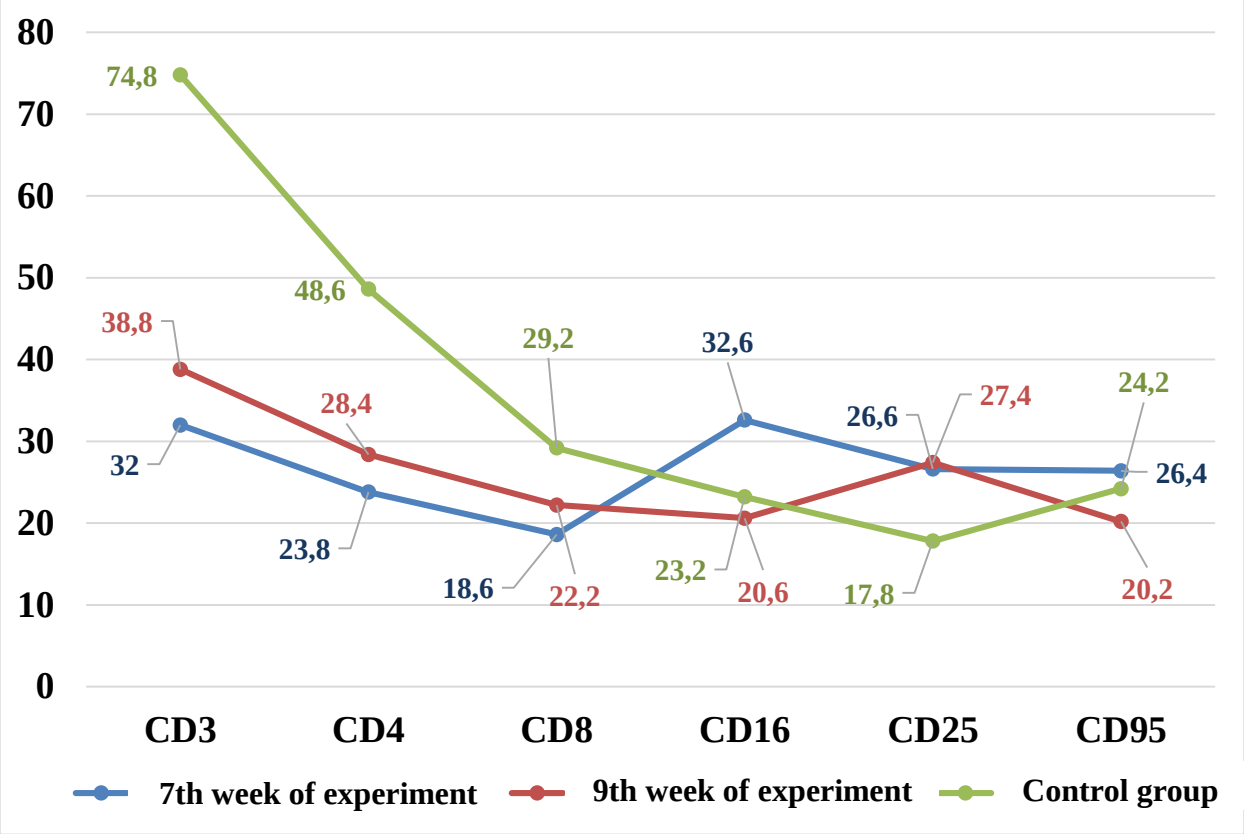


Fig. 3. Dynamics of changes in immunological

blood parameters of the 1st group

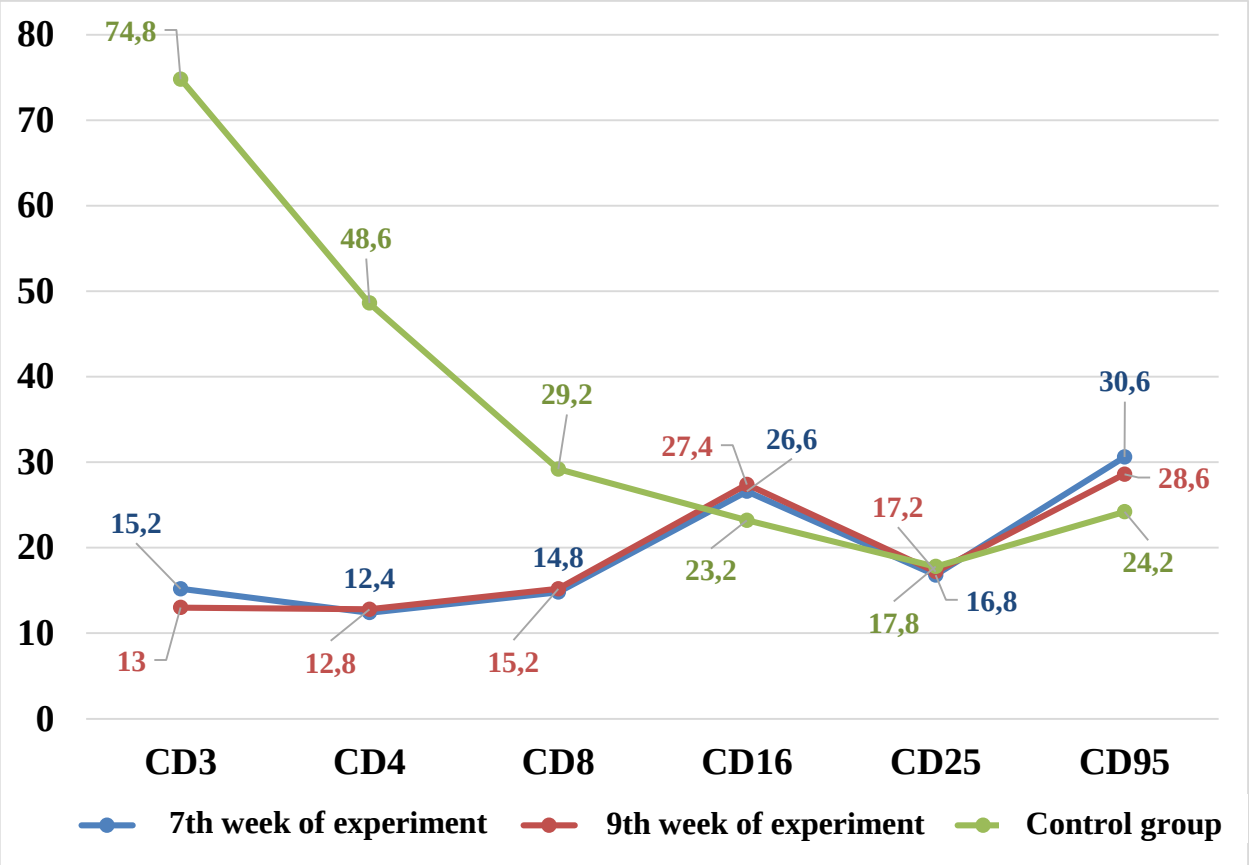


Fig. 4 Dynamics of changes in immunological blood parameters of the 2nd group

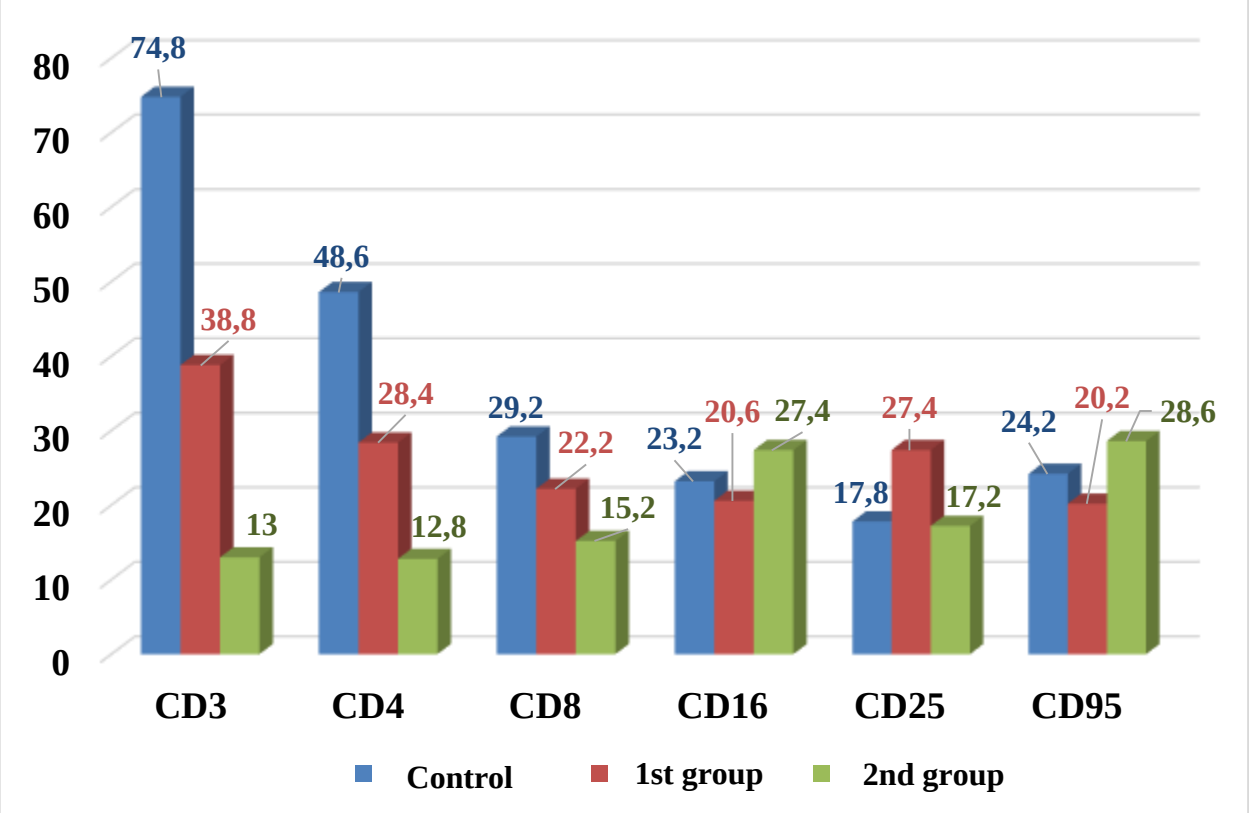


Fig. 5 Immunological parameters of blood on the 9th week of the experiment in the comparison groups

Results of histological studies of the thymus: The thymus capsule is formed by dense fibrous connective tissue, which from connective tissue septa is extended, dividing the gland into lobules. The basis of the thymus parenchyma is formed by epithelial cells (epithelia-reticulocytes). Epithelial cells also form layered epithelial corpuscles of thymus - Hassall corpuscles, "epithelial pearls" in the medulla. Research has shown a decrease in the number of lymphocytes. The medulla is less cellular. Hypolymphotization of the lobules. Migration of thymic bodies to the periphery of the lobules (Fig. 6-7).

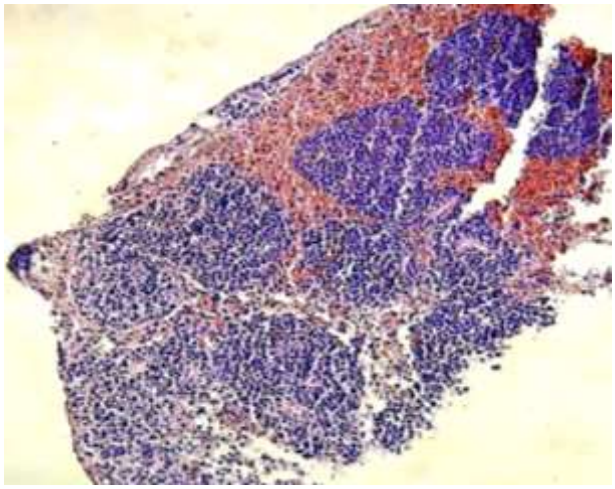


Fig. 6. Thymus. Mag. X10. Hematoxylin-Eosin staining.

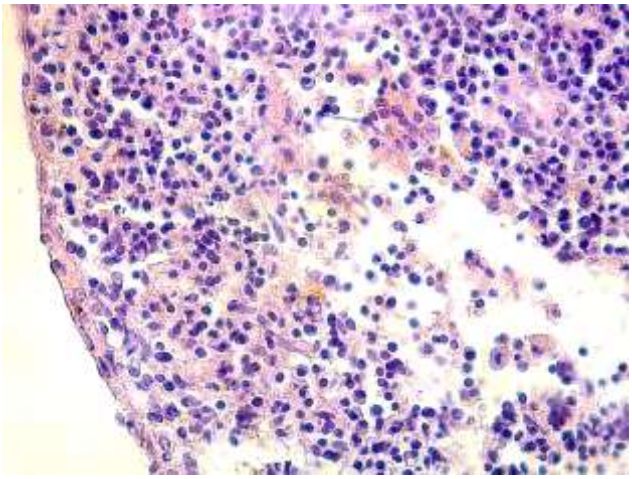


Fig. 7. Thymus. Mag. X40. Hematoxylin-Eosin staining.

Morphological examination of the autoimplant on the 9thweek of the experiment revealed a partial, cellular dysplasia of the thymus (autoimplant). Thymic hyperplasia with plasma cells in the dilated perivascular spaces and a decrease in thymic cortex with accumulation of erythrocytes were also found. In 30% of cases, thymus involution was established with replacement of its parenchyma with adipose connective tissue. In 35% of cases, the proliferation of fibrous collagen connective tissue was revealed (Figures 5,8,10,11). This is undoubtedly related to the age of the animals.

It is known that before the age of puberty, the size and number of lobules increase, later they stabilize and the process of age-related involution begins. In rabbits of this breed, they reach sexual maturity by about 5-6thmonths of age [6]. Observation of the process of involution in an autoimplant in experimental rabbits at 5.5thmonths of age (30%) proves its functioning.

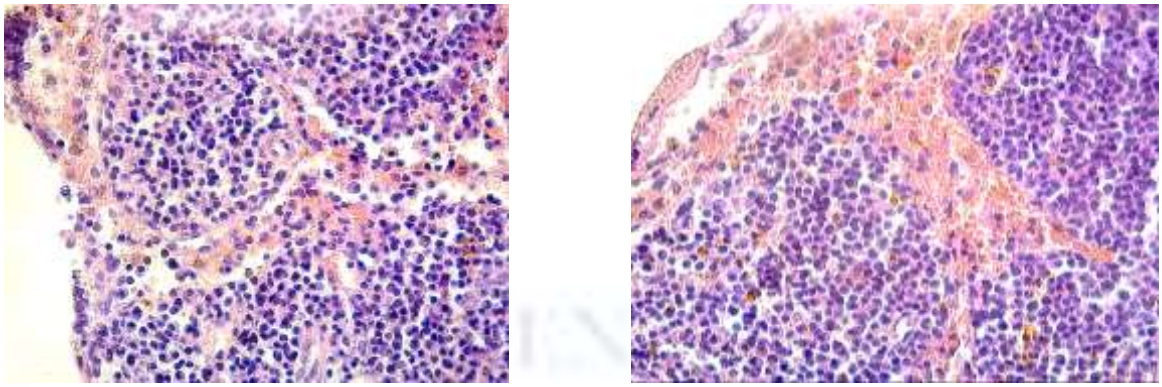


Fig. 8-9. Thymus (autoimplant). Mag. X40. Coloring hematoxylin - Eosin

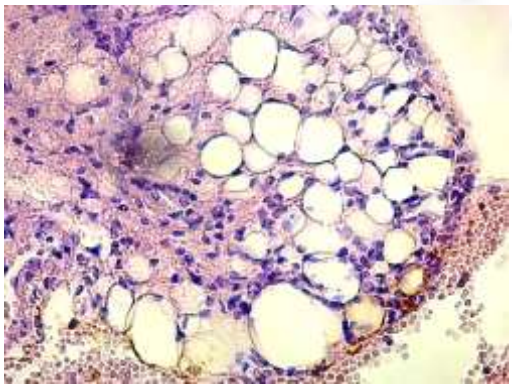


Fig. 10. Thymus (autoimplant). Mag. X40. Coloring hematoxylin - Eosin.

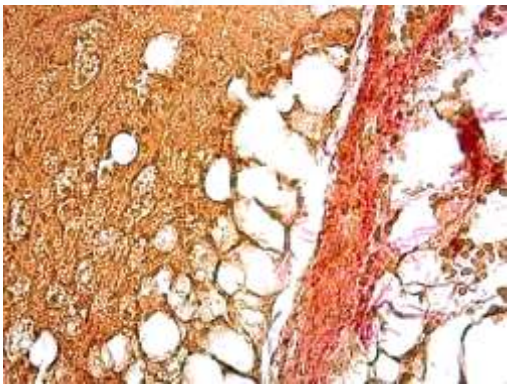


Fig. 11. Thymus (autoimplant). Mag. X40. Staining according to Van Gieson.

Conclusion: The clinical and immunological effects of the influence of autoimplantation on the stages of experimental research have been established.

Histomorphological processes in the thymus withmedian sternotomy with thymectomy for surgical correction of congenital heart disease showed depletion of the morphofunctional status of the organ, associated with increasingreducing its potential. During the first 9 weeks of observation after

thymectomy, severe anemia, erythrocytopenia, leukopenia, accelerated ESR up to 20 mm / h, hypoproteinemia, hypoalbuminemia, developed uremia.

Therefore, thymectomy is an unfavorable factor.for the activity of the entire immune system of the body, which must be taken into account to predict the effects of the formation of adaptive immunity and the development of postoperative complications.

At autoimplantation in the thymus develops a complex of morphological changes that affect the quality of the intrathymic development of *T*-lymphocytes and, as a result, the preservation of the *T*-cell resource in the body. It was found that in the blood of rabbits during 9 weeks of autoimplantation of the thymus, the number of CD3 + *T*-cells is 2.98 times higher (up to 38.8 ± 3.17%), CD4 + lymphocytes are 2.21 times (28.4 ± 3.36%)and CD8 + lymphocytes 1.46 times (22.2 ± 22.17%), than in rabbits with thymectomy, respectively, 13 ± 2.97%; 12.8 ± 0.84%; 15.2 ± 1.1% ($p < 0.01$).

Thus, autoimplantation of the thymus helps restore immunity and improve the quality of life during surgical correction of congenital heart disease.

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