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**PROCEEDINGS OF VII INTERNATIONAL  
SCIENTIFIC AND PRACTICAL CONFERENCE  
MARCH 26-28, 2026**

**VANCOUVER  
2026**

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# **MEDICAL SCIENCES**

## **ALTERATIONS IN RICINUS COMMUNIS AGGLUTININ (RCA) RECEPTOR EXPRESSION ON THYMOCYTES UNDER NORMAL CONDITIONS AND FOLLOWING PRENATAL ADMINISTRATION OF STAPHYLOCOCCAL TOXOID IN RATS**

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**Introductions:** The thymus is a key organ in the system of immunoendocrine regulation, functioning both as an immune organ and an endocrine gland, and performing essential roles in ensuring the structural and functional maturation of immunocompetent cells. Immunoendocrine interactions of the thymus with other organs are of particular importance in early childhood, as they support normal development, maintain immune reactivity, and facilitate adaptation to various environmental factors. Disruption of thymic development may underlie the pathogenesis of autoimmune, allergic, and infectious diseases in young children. It is generally assumed that impaired formation and maturation of the fetal immune system are associated with its suppression due to excessive antigenic stimulation by infectious agents and other foreign antigens of maternal origin, particularly under conditions of compromised placental barrier function. Notably, the migration of maturing thymocytes within the thymus is governed both by chemokine gradients produced by stromal cells and by the expression of corresponding receptors on the

surface of thymocytes. Lymphocytes express a variety of glycoconjugates on their surface membrane, which allows lectins to be used for the characterization of lymphocyte properties. Ricinus communis agglutinin (RCA) exhibits specificity for  $\beta$ -D-galactose residues masked by sialic acid. Sialoconjugates, which serve as receptors for RCA lectins, determine the migratory activity of lymphocytes..

**Aim:** To study the dynamics of ricinus communis agglutinin lectin (RCA) receptor expression on thymocytes in normal conditions and after intrauterine exposure to staphylococcal anatoxin in rats.

**Materials and methods:** The studies were conducted on 144 white rats on postnatal days 1, 2, 3, 5, 9, 14, 21, and 30. The animals were divided into three groups (n = 48 per group): (1) an intact group (Int); (2) an experimental group (SA), in which 0.05 mL of staphylococcal toxoid was administered on gestational day 18 via intrauterine, transmembrane, intrafetal injection; and (3) a control group (K), which received 0.05 mL of 0.9% NaCl solution.

For the identification of  $\beta$ -D-galactose carbohydrate residues masked by sialic acid, expressed by RCA<sup>+</sup> lymphocytes, ricinus communis agglutinin (RCA) lectin was used.

For this purpose, serial histological sections were preliminarily deparaffinized in xylene and passed through 96% ethanol and a methanol–hydrogen peroxide solution (1:9), followed by rehydration in descending concentrations of ethanol (60% and 30%). The sections were rinsed in phosphate-buffered saline (PBS) and subsequently incubated with the corresponding lectin solution at a dilution of 1:50 for 45 minutes in a humid chamber at +37°C.

After removal of excess lectin solution by washing in PBS, the sections were incubated for 15 minutes in a humid chamber at +37°C with a solution of 3,3'-diaminobenzidine (DAB) to visualize lectin-binding sites. The sections were then rinsed in 40% ethanol to remove excess DAB, dehydrated in absolute ethanol and xylene, and mounted in balsam.

Specificity of the reaction was controlled by incubating the sections in 1% periodic acid (HIO<sub>4</sub>) for 30 minutes in a humid chamber.

The prepared histological sections were analyzed and photographed within a standardized area of 1500  $\mu\text{m}^2$ . Lectin receptor expression was assessed using a semi-quantitative scoring system (from + to +++). The intensity of DAB deposition was graded as follows: +++ — strong reaction (brown staining); ++ — moderate reaction (yellow-brown staining); + — weak reaction (light-brown staining); 0 — no reaction.

**Results and discussion:** The introduction of saline to the K group of animals did not cause significant changes ( $p < 0.05$ ) in the thymus compared to the Int group at all studied periods.

In animals of the intact (Int) and control (K) groups, a low (+) intensity of receptor expression for ricinus communis agglutinin (RCA) was observed in the thymic cortex throughout the entire observation period, with the exception of day 2 after birth, when this parameter increased to a moderate level (++) . In contrast, in the experimental (SA) group of animals, the intensity of DAB deposition corresponding to  $\beta$ -D-galactose residues masked by sialic acid, in cortical substance of thymus, was maintained at a moderate (++) level up to and including day 5 of postnatal life, after which a decrease in this parameter to a low (+) level was recorded until the end of the observation period.

In the thymic medulla, the expression of  $\beta$ -D-galactose-glycoconjugates on the surface membrane of lymphocytes was observed at a moderate (++) level on day 1 of life, and was high (+++) on day 2 after birth in all studied groups, with no significant differences between them.

On day 3 of the postnatal period, in the thymic medulla of the intact (Int) and control (K) groups of animals, the density of receptors for  $\beta$ -D-galactose residues masked by sialic acid decreased, with a moderate level of expression (++) observed. In SA group of animals, the density of distribution of ricinus communis agglutinin (RCA) receptors remained at a high (+++) level.

On day 5 after birth, in the thymic medulla, a moderate (++) level of RCA<sup>+</sup> receptor expression was observed in all studied groups.

Subsequently, in the thymic medulla of animals in the intact (Int) and control (K) groups, from day 9 to day 30 inclusive, the intensity of DAB deposition

corresponding to ricinus communis agglutinin (RCA) decreased and was recorded at a low (+) level.

Meanwhile, in the experimental (SA) group, the density of DAB deposition corresponding to  $\beta$ -D-galactose residues masked by sialic acid from day 9 until the end of the observation period remained at a moderate (++) level, except on day 14, when a decrease of this parameter to a low (+) level was recorded.

**Chart 1**

**Distribution of ricinus communis agglutinin (RCA) receptors in the cortical region of the thymus of rats during the postnatal period**

Day	Int	SA	K
1st	+	++	+
2nd	++	++	++
3rd	+	++	+
5th	+	++	+
9th	+	+	+
14th	+	+	+
21st	+	+	+
30th	+	+	+

Note: Int – intact group, K – control group, SA – experimental group of animals administered staphylococcal toxoid.

**Chart 2**

**Distribution of ricinus communis agglutinin (RCA) receptors in the medullary region of the thymus of rats during the postnatal period**

Day	Int	SA	K
1st	++	++	++
2nd	+++	+++	+++
3rd	++	+++	++
5th	++	++	++
9th	+	++	+
14th	+	+	+
21st	+	++	+
30th	+	++	+

Note: Int – intact group, K – control group, SA – experimental group of animals administered staphylococcal toxoid.

**Conclusions:** In the Int group of animals, the intensity of Ricinus communis agglutinin (RCA) receptor expression exhibited a surge in lymphocyte migratory activity from the thymic medulla to the periphery during the first days after birth, followed by a balancing of maturation and migration processes.

In the group of animals subjected to prenatal administration of staphylococcal toxoid, a more intense migratory activity of thymocytes was observed in all thymic compartments, as well as a longer duration of this process, particularly in the thymic medulla.

The above-mentioned features in the experimental group likely reflect antigen-mediated alterations in the migratory properties of thymocytes, acceleration of lymphocytes maturation processes, as well as their earlier egress to the periphery, which may constitute a basis for the development of pathological conditions.