AJPMHS GLOBAL RESEARCH NETWORK

AMERICAN Journal of Pediatric Medicine and Health Sciences
Volume 2, Issue 3, 2024 ISSN (E): 2993-2149

Characteristics of Laboratory Animals Involved in the Experiment

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Relevance of the study. The thymus (thymus gland) is a large lymphoid organ. The mass of the thymus can reach 37-40 g by adulthood. Maturation and subsequent differentiation of the largest population of T–lymphocytes (thymus-dependent lymphocytes) takes place in this organ. Structurally, the thymus consists of large lobes covered with a connective tissue capsule. Flat partitions extending from the capsule into the thymus divide the thymus tissue into small segments 1-2 mm in size. The lobule is a structural unit of the thymus. The main mass of thymus lymphocytes (cortical zone) is located on the periphery of the lobule. Towards the center, the concentration of lymphocytes is lower (the brain area). Progenitor cells (medium lymphocytes, lymphoblasts), which came with the blood flow from the bone marrow, are localized on the periphery of the thymus lobule and actively divide, forming many small lymphocytes. Maturing, lymphocytes enter the medulla, from where mature T cells enter small venous vessels and are carried with the blood flow throughout the body. The thymus gland is located directly behind the handle of the sternum. Due to the topography of the thymus gland in congenital heart defects, a thymectomy is performed to improve the visualization of heart structures [1.3.5].

It is known that the thymus is the central organ of the immune system, where the maturation of T-lymphocytes and the formation of central tolerance take place. From these positions, the removal of the thymus, especially during the periods of newborn or infancy, should be critical for the formation of the T-cell link of the immune system in the postnatal period. The immune system is a child's adaptation system to various environmental factors. The functioning of the immune system, including the thymus gland, also depends on the quality of organogenesis of these organs.

The human thymus is laid at the beginning of the second month of development in embryos from two sources: ecto- and endoderm of the ventral walls of the third pharyngeal pockets and slits in the form of two separate rudiments (future right and left lobes of the organ), which approach as closely as possible by the middle of the second month of prenatal ontogenesis. By the middle of the 3rd month of human intrauterine development, the thymus acquires a definitive structure: lobules, cortical and cerebral matter are formed in its lobes and thymus corpuscles are found. The growth of the thymus in prenatal human ontogenesis is characterized by a wave–like course, there are three periods of accelerated growth: the first – in the middle of the second month (associated with the ingrowth of vessels into the thymus and the colonization of the epithelial lining of the organ by lymphoid cells), the second - at the end of the 3rd month (associated with the completion of organogenesis), the third – 6-8 months development (corresponds to the active growth of the fetus [2.4.6].

The quality of organogenesis is important from the point of view of assessing the functions of the thymus gland in the early stages of a child's development (last trimester, newborn period, first three years of life), especially during periods of the greatest activity of the thymus gland. The thymus reaches its maximum size by puberty, when its body weight reaches an average of 37.5 g (10-15 years). At 16-20 years of age, the mass of the thymus averages 25.5 g, and at 21-

35 years of age 22.3 g, at 50-90 years of age - 13.4 g. The lymphoid tissue of the thymus does not completely disappear even in old age, remaining in the form of separate islands (lobules) separated by adipose tissue. The highest production of T-lymphocytes persists for up to two years. It is during these years that primary contacts with infectious agents occur and long-lived memory T cells are formed, which live for more than 20 years and reproduce themselves. In the future, the arrival of new pathogens becomes a rarer event, in connection with which the maintenance of the whole thymus by the body becomes impractical and the thymus undergoes age-related involution at a rate of ~ 3% per year of true thymic tissue.

The pool of mature peripheral T lymphocytes created with high energy costs (subsequently migrating from the thymus to tissues) includes relatively long-lived cells capable of responding with clonal expansion (proliferation) to an antigen encounter. Therefore, age-related thymus involution does not lead to a catastrophic decrease in immunity. In addition, the immune system has some compensatory possibilities for replacing certain functions of missing T-lymphocytes.

The purpose of the research. To identify the role of the thymus gland in the mechanisms of regulation of the normal microflora of the large intestine as the central organ of the immune system and to perform biocorrection to prevent the occurrence of intestinal dysbiosis after thymectomy.

Research objectives. To achieve the main goal, it is planned to solve the following tasks: (no more than 5 tasks)

- to study the normal microflora of the large intestine in intact (control group) and falsely operated rats;
- perform a thymectomy in the main group;
- to identify changes in the microflora of the large intestine in thymectomized rats;
- describe the quantitative and qualitative characteristics of the changes detected in thymectomized rats;
- to make a comparative characteristic in thymectomized and control group rats;

Results and analyzes. To study the effect of thymectomy on the state of the colon microflora, experimental studies were conducted on laboratory animals (white mongrel rats). 135 white mongrel rats, 3 months old, weighing 160-180 g, were involved in the research, which were divided into the following groups.

The main group were white mongrel rats who underwent thymectomy and after 1, 3, 6 months, the normal microflora of the colon was studied (n=60);

The comparison group was white mongrel rats, in which thymectomy was not performed, but the normal microflora of the colon was studied - “falsely operated" (n=60);

In adult rats, thymectomy is associated with 20% mortality and often leads to incomplete thymectomy. Thymectomy in adult rats is complicated in the form of hemorrhage and pneumothorax due to rupture of the pleura can lead to significant mortality. This method (Victoria R. Rendell et al., 2014) is a simple rat thymectomy method that uses mini sternotomy and endotracheal intubation. Intubation is performed in a non-invasive and easily reproducible method and allows positive pressure ventilation to prevent pneumothorax and controlled airways, which gives enough time for careful dissection of the thymus to minimize damage to the pleura. A 1.5 cm long sternum incision reduces contact with mediastinal vessels and pleura, while providing full visualization of this organ. After the mediastinum is exposed, it is removed by blunt dissection under magnification. Then the pleural cavity is sealed by suturing the pretracheal muscles, followed by applying surgical glue. Then the chest is sewn up by suturing the sternum, followed by suturing the skin. The survival rate was 90%.

Apply providon-iodine to the chest area. Then clean the surface of the skin with 700 ethyl alcohol on gauze. Cover the rat with a transparent plastic film that cuts a hole to expose the sterile surgical field.

It is necessary to determine the suprasternal notch in the upper thoracic region, make a median skin incision 2 cm long, starting 2-3 mm above the suprastern notch and continuing distally along the median line along the sternum using Shea scissors with blunt ends. A median sternotomy is performed 1.5 cm from the suprastern notch again using Shea scissors with blunt ends. Hold the bottom edge of the scissors directly under the sternum and slowly move forward. Insert a small retractor Alm directly under the separated sternum and open to reveal the pretracheal band muscles - the thoracophyloid and sternocyroid muscles. To separate the pretracheal muscles of the belt with the help of blunt Grefe forceps. Place the pins of the small Alma retractor under the separated belt muscles and sternum. Open the retractor to expose the upper aspect of the thymus gland. Use thin Dumont forceps to release the lateral edges of the thymus gland tissue and expose the lower lobes of the thymus. It is necessary to pull the thymus upward into the open area of the incision, trying to avoid contact with the superior vena cava, subclavian and carotid vessels and minimize the violation of the thin pleural membrane between the thymus and the lungs. Thymectomy includes a 1.5 cm sternotomy and a three-layer closure using surgical glue to seal the mediastinum and minimize the frequency of bleeding and respiratory complications. This method reliably leads to a complete thymectomy. The duration of the operation and periprocedural mortality are minimized.

The control group consisted of intact white mongrel rats (n=15). The main and comparative groups, in turn, were divided into 3 more subgroups - the results of the study of the normal microflora of the colon after 1 month (O1), 3 months (O2) and 6 months (O3) after thymectomy, 1 month (C1), 3 months (C2) and 6 months (C3) after the “false operation".All laboratory animals were taken from the same vivarium and were of the same age. They were kept in plastic cages under standard vivarium conditions with relative humidity (50-60%), temperature (19-22 °C) and lighting mode (12 hours in dark and light mode, respectively). Passport data was hung in every cage where white mongrel rats were kept. To make sure that there were no infections in the vivarium, laboratory animals were quarantined for 21 days and monitored during these days, their body temperature was measured several times during these days and their weight was monitored, during the examination they were monitored for weight gain. During this period, they did not have any symptoms of the disease, and their temperature was within the normal range (38.5 - 39.5 oC). The corpses of animals that died during the experiment were buried in the soil during the disposal process, having previously issued the appropriate documents (act), the corpses of white mongrel rats were treated with a 20% solution of bleach before burial [6.7.8.9].

Conclusion. The main focus was on the randomization of studies in compliance with the principles of evidence-based medicine. The study strictly followed the ethical principles of working with laboratory animals and the rules of biological safety.

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