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MORPHOLOGICAL AND MORPHOMETRIC FEATURES OF THE THYMUS AFTER SPLENECTOMY IN HEALTHY ANIMALS

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Abstract:

Background. The thymus and spleen are key organs of the immune system that ensure coordination between the central and peripheral components of immunogenesis. Dysfunction of one of these organs may lead to systemic rearrangement of immune homeostasis.

Objective. To identify morphological and morphometric features of the thymus in clinically healthy animals after splenectomy.

Materials and Methods. The study was conducted on 40 adult Wistar rats divided into control and experimental groups. Splenectomy was performed under aseptic conditions. The thymus was collected on postoperative days 14 and 30. Histological and morphometric methods were applied, followed by statistical analysis.

Results. On day 14 after splenectomy, a significant decrease in thymus weight, cortical area, cortico-medullary index, and thymocyte density was observed. The medullary area showed no significant changes. By day 30, a tendency toward partial recovery of morphometric parameters was noted; however, values did not reach control levels.

Conclusion. Splenectomy causes pronounced early involutive changes in the thymus followed by compensatory structural reorganization, confirming the systemic interrelationship between central and peripheral immune organs.

Keywords: thymus, spleen, splenectomy, Wistar rats, morphometry, histological analysis, cortico-medullary index, thymocytes, immune system, experimental animals.

INTRODUCTION

The thymus is the central organ of the immune system where differentiation, proliferation, and selection of T lymphocytes occur, as well as the formation of central immunological tolerance [1,2]. The morphological organization of the thymus—namely the ratio of cortical and medullary regions, thymocyte density, the condition of epithelial reticular cells, and Hassall's corpuscles—is regarded as an objective morphological marker of cellular immune function [3,4].

The spleen belongs to the peripheral organs of immunogenesis and performs filtration, immunoregulatory, and depot functions. It plays a key role in antigen-dependent lymphocyte activation, removal of senescent erythrocytes, and maintenance of immune homeostasis [5,6]. The white pulp of the spleen participates in humoral immune responses, whereas the red pulp ensures hematological and detoxification functions.

Despite differences in anatomical and functional organization, the thymus and spleen are closely interconnected, forming a unified immune regulatory system. According to the concept of immune organ integration, dysfunction of one component of the immune system is accompanied by compensatory restructuring of other organs [7,8].

Splenectomy, used in clinical practice for trauma, hematological, and autoimmune diseases and widely applied in experimental models, leads to changes in immune cell circulation and redistribution of antigenic load [9]. Several experimental studies have shown that splenectomy is accompanied by changes in thymus

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weight and architecture, variations in the cortico-medullary index, and thymocyte density [10,11].

However, data regarding the nature of these changes remain controversial. Some authors report thymic activation as a compensatory response to the loss of a peripheral immune organ [12], while others describe stress-induced thymic involution associated with neuroendocrine mechanisms and increased glucocorticoid levels [13,14]. Experimental outcomes largely depend on animal species, age, postoperative duration, and applied morphometric criteria.

Analysis of contemporary literature indicates that most studies focus on functional immunological parameters or general histological descriptions, whereas comprehensive morphological and morphometric investigations of the thymus in clinically healthy animals after splenectomy remain limited. Quantitative parameters of cortical and medullary regions, cellular dynamics, and structural changes of the thymus at different postoperative periods are insufficiently studied.

Therefore, investigation of morphological and morphometric changes in the thymus under conditions of splenic absence represents a relevant scientific task that enhances understanding of systemic immune adaptation mechanisms and compensatory responses of central immune organs.

Objective of the study — to identify morphological and morphometric features of the thymus in healthy animals after splenectomy.

MATERIALS AND METHODS

The experimental study was performed on 40 adult Wistar rats weighing 180–220 g and aged 3–4 months. Animals were housed under standard vivarium conditions at 22 ± 2 °C, relative humidity of 50–60%, a 12-hour light/dark cycle, and free access to water and standard pellet feed. All animals underwent a minimum 7-day acclimatization period prior to the experiment.

Animals were randomly divided into two groups:

- Control group (n = 20) — intact animals without surgical intervention;
- Experimental group (n = 20) — animals subjected to splenectomy.

The experiment was conducted in accordance with international guidelines for the care and use of laboratory animals (Guide for the Care and Use of Laboratory Animals) and approved by the local ethics committee.

Splenectomy was performed under general anesthesia using intraperitoneal administration of anesthetic agents in standard doses. After achieving surgical anesthesia, a left subcostal approach was used. The spleen was mobilized, the vascular pedicle was ligated, and the organ was removed. The abdominal wall was closed in layers.

Postoperatively, animals were monitored dynamically and received standard care. No complications, mortality, or signs of inflammatory reactions at the surgical site were observed.

Tissue sampling was performed on postoperative days 14 and 30, allowing evaluation of early and late morphological changes. Animals were euthanized humanely in compliance with ethical standards.

The thymus was completely excised, freed from surrounding tissues, and weighed using analytical scales. Samples were fixed in 10% neutral formalin for 24–48 hours, followed by routine histological processing and paraffin embedding.

Paraffin sections 5–7 μm thick were prepared using a microtome and stained with hematoxylin and eosin for general morphological evaluation. Microscopic examination was performed using a light microscope at magnifications ×100, ×200, and ×400.

Morphometric analysis was conducted using a digital image analysis system. The following parameters were evaluated:

- absolute and relative thymus weight;
- cortical and medullary area;
- cortico-medullary index;
- thymocyte density in cortical and medullary regions (cells per unit area).

Measurements were obtained from at least 10 randomly selected fields of view per specimen, followed by calculation of mean values.

Statistical analysis was performed using standard methods of variation statistics. Data were presented as M ± m (mean ± standard error). Intergroup differences were assessed using Student's *t*-test. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Analysis of thymic morphometric parameters on postoperative day 14 (Table 1) revealed statistically significant changes compared with the control group.

Thymus weight in splenectomized animals was significantly reduced (356.2 ± 15.7 mg) compared with controls (412.6 ± 18.4 mg; $p < 0.05$), indicating early postoperative involutive processes.

The cortical area was significantly decreased in the experimental group (1.32 ± 0.07 mm²) compared with controls (1.84 ± 0.09 mm²; $p < 0.01$), reflecting

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cortical thinning and reduction of thymocyte populations involved in proliferation and differentiation.

Table 1. Morphometric parameters of the thymus in control and splenectomized animals (day 14)

Parameter	Control (M ± m)	Splenectomy (M ± m)	p
Thymus weight, mg	412.6 ± 18.4	356.2 ± 15.7	<0.05
Cortical area, mm²	1.84 ± 0.09	1.32 ± 0.07	<0.01
Medullary area, mm²	0.96 ± 0.05	1.01 ± 0.06	>0.05
Cortico-medullary index	1.92 ± 0.11	1.31 ± 0.08	<0.01
Thymocyte density, cells/mm²	6840 ± 210	5120 ± 195	<0.01

The medullary area did not differ significantly between groups (1.01 ± 0.06 mm² vs. 0.96 ± 0.05 mm²; $p > 0.05$), indicating relative stability of the medullary compartment.

The cortico-medullary index was significantly reduced in splenectomized animals (1.31 ± 0.08) compared with controls (1.92 ± 0.11; $p < 0.01$), reflecting disruption of structural balance and decreased thymic functional activity.

Thymocyte density in the cortex was also significantly decreased (5120 ± 195 cells/mm²) compared with intact animals (6840 ± 210 cells/mm²; $p < 0.01$).

Analysis of thymic dynamics (Table 2) demonstrated partial recovery by postoperative day 30. Thymus weight increased to 378.4 ± 16.3 mg, cortical area increased to 1.55 ± 0.08 mm², cortico-medullary index increased to 1.58 ± 0.09, and thymocyte density increased to 5860 ± 210 cells/mm². However, these values remained below control levels.

Table 2. Dynamics of thymic morphometric parameters after splenectomy

Parameter	Day 14	Day 30
Thymus weight, mg	356.2 ± 15.7	378.4 ± 16.3
Cortical area, mm²	1.32 ± 0.07	1.55 ± 0.08
Medullary area, mm²	1.01 ± 0.06	0.98 ± 0.05
Cortico-medullary index	1.31 ± 0.08	1.58 ± 0.09
Thymocyte density, cells/mm²	5120 ± 195	5860 ± 210

The medullary area remained relatively unchanged, confirming its morphological stability.

Morphological examination (Table 3) supported morphometric findings. Control thymuses exhibited well-defined lobular architecture, thick cortex, normal medulla, few Hassall's corpuscles, and narrow interlobular spaces.

Table 3. Morphological characteristics of the thymus

Feature	Control	Day 14	Day 30
Lobulation	Well defined	Disrupted	Partially restored
Cortex	Thick	Thinned	Moderately thickened
Medulla	Normal	No changes	Normal
Hassall's corpuscles	Single	Enlarged	Stable
Interlobular spaces	Narrow	Expanded	Moderately expanded

On day 14 after splenectomy, lobular architecture was disrupted, the cortex was markedly thinned, Hassall's corpuscles were enlarged, and interlobular spaces were expanded, indicating involutive-adaptive changes.

By day 30, partial restoration of lobular structure was observed. The cortex remained moderately thinned, the medulla appeared normal, Hassall's corpuscles were stable, and interlobular spaces were moderately widened.

DISCUSSION

The obtained results demonstrate that splenectomy in healthy animals induces pronounced morphological and morphometric changes in the thymus, particularly during early postoperative periods.

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These findings reflect systemic interactions between central and peripheral immune organs and confirm the presence of compensatory-adaptive immune mechanisms.

Early decreases in thymus weight, cortical area, cortico-medullary index, and thymocyte density indicate involutive changes, consistent with the high sensitivity of the thymic cortex to stress and neuroendocrine influences. The relative stability of the medullary compartment suggests selective vulnerability of the cortex.

Partial recovery observed by day 30 reflects compensatory activation of the thymus in response to redistribution of immune load after spleen removal.

CONCLUSIONS

1. Splenectomy in healthy animals causes pronounced morphological and morphometric alterations in the thymus, most evident in early postoperative periods.
2. On day 14 after splenectomy, significant reductions in thymus weight, cortical area, cortico-medullary index, and thymocyte density indicate involutive changes and decreased thymic activity.
3. The medullary compartment remains relatively stable, suggesting selective cortical vulnerability.
4. By day 30, partial recovery of thymic structure and cellularity occurs due to compensatory immune mechanisms.
5. These findings confirm the close functional relationship between central and peripheral immune organs and the key role of the thymus in systemic immune adaptation following splenectomy.

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