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Relationship between esophageal morphology in rabbits and autonomic regulation type

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The morphofunctional characteristics of the organism as a whole and of individual organs in particular depend on regulatory influences of the autonomic nervous system. The balance between sympathetic and parasympathetic divisions determines a specific autonomic regulation type. The influence of this factor on esophageal anatomy was investigated in male Blanc de Termonde rabbits. Each animal underwent electrocardiographic examination to determine autonomic tone. After euthanasia, histological sections were prepared from the thoracic esophagus, and the areas of the esophageal wall layers and their components were measured. Rabbits with high sympathetic tone showed increased areas of the mucosa (epithelium, keratinized layer, and lamina propria) and the submucosa. The epithelial area in the rabbits with high sympathetic tone (1.925 ± 0.050 mm²) exceeded that in the rabbits with high parasympathetic tone by 0.221 mm² (P = 0.0076). The rabbits with high parasympathetic tone had larger areas of the outer longitudinal, circular, and inner longitudinal layers of the muscular layer. The total area of muscular membrane in the rabbits with high parasympathetic tone (9.977 ± 0.281 mm²) exceeded that in the rabbits with balanced autonomic tone by 1.465 mm² (P = 0.0430). For all types of autonomous regulation, the muscular membrane showed numerous correlations with other structural components of the esophageal wall. The rabbits with balanced autonomic tone were characterized by the largest areas of the serosa and the muscularis mucosae. The muscularis mucosae area was lowest in the rabbits with high parasympathetic tone (0.661 ± 0.018 mm²), which was 0.089 mm² smaller than in the rabbits with balanced autonomic tone (P = 0.0151). Autonomic regulation type was also reflected in the proportions of esophageal wall components (mucosa, submucosa, muscular membrane, serosa). The ratio was 20.98% – 12.64% – 62.06% – 4.32% in the rabbits with high sympathetic tone, 21.73% – 10.22% – 61.10% – 6.95% in the rabbits with balanced autonomic tone, and 19.08% – 10.18% – 67.34% – 3.40%. in the rabbits with high parasympathetic tone. High parasympathetic tone was associated with the lowest variability of most parameters, and similar variability was observed in the rabbits with high sympathetic tone. By contrast, the rabbits with balanced autonomic tone showed a marked increase in the coefficient of variation.

Keywords: autonomic regulation type; sympathetic tone; parasympathetic tone; esophageal wall; morphometry.

Introduction

The development of the esophagus during ontogenesis occurs under the control of the autonomic nervous system, which, on the one hand, directly influences organ function (Richards & Sugarbaker, 1995) and, on the other, regulates tissue perfusion via the cardiovascular system (Taylor et al., 2014). Autonomic nerves innervating the rabbit esophagus form two extensive plexuses: one located externally to the organ and another within its wall (Cecio & Califano, 1967). Adrenergic nerve fibers are most densely distributed around blood vessels and within the intermuscular nerve plexus (Nishimura & Takasu, 1969; Khamraev, 2023). Motor functions of the esophageal wall are mediated by vagal influences (Park & Conklin, 1999).

The integration of neural elements with all cellular and tissue components of the esophageal wall determines the complex neurophysiology of this organ in normal conditions (Woodland et al., 2013; Nikaki et al., 2019) and its nociception in pathology (Brock et al., 2016).

The esophageal wall in rabbits, as in other mammals, consists of the mucosa with submucosa, the muscular membrane, and either adventitia (in the cervical region) or serosa (in the thoracic and abdominal regions) (Ranjan & Das, 2016). Age-related changes are most pronounced in the mucosa and are associated with progressive epithelial keratinization, the presence of the muscularis mucosae (Selim et al., 2017), and its relationship with the muscular membrane (Hughes, 1955). The latter thickens in the caudal direction (Cecio, 1976). For improved visualization of esophageal structure, *in vitro* 3D models have been developed, including Organotypic 3D culture and Spheroid culture (Stanforth et al., 2022).

Investigation of rabbit esophageal morphology is justified by its importance as a biological model for experimental research (Mapara et al., 2012; Glenn et al., 2017), evaluation of novel therapeutic approaches (Song et al., 2023; Adly et al., 2024), and acquisition of technical skills and operative experience in abdominal and thoracic endosurgery (Kirlum et al., 2005). In particular, the rabbit esophagus is used in studies of the adverse effects of various types of stress (Tobey et al., 1999; Ergün et al., 2021), the course of gastroesophageal reflux disease (De Hertogh et al., 2006; Hu et al., 2013), and the harmful effects of alcohol (Bor & Capanoglu, 2009) and cigarette smoke (Orlando et al., 1986).

The most common approach to studying esophageal structure is measurement of the thickness of the wall and its individual layers (Mohammad et al., 2020; Cipou et al., 2021). We previously conducted a similar study taking into account autonomic regulation type (Tybinka & Zakrevska, 2025) and found that mucosal thickness is heterogeneous and increases two- to threefold in the regions of folds, which reduces measurement objectivity. To overcome this limitation, we measured the areas of structural components of the esophageal wall (Tybinka et al., 2025), but without considering autonomic regulation characteristics. Investigation of this factor therefore became the objective of the present study.

Materials and methods

The study was conducted on clinically healthy male rabbits (*Oryctolagus cuniculus* [Linnaeus, 1758]) of the Blanc de Termonde breed, aged four months and weighing 3.6–3.9 kg. Individual characteristics of autonomic regulation were determined in each animal using electrocardiographic assessment of the heart rate variability. Cardiac signals were recorded at a paper speed of 250 mm/s. Electrocardiograms were analyzed using a geometric method of R–R interval assessment with determination of the following parameters: mode (Mo), i.e. the most frequent R–R interval; mode amplitude (AMo), i.e. the percentage of R–R intervals forming the mode; and variation range (MxDMn), i.e. the difference between the longest (Mx) and shortest (Mn) R–R intervals.

Based on these parameters, three autonomic regulation types were identified, and the animals were divided into three groups (n = 5 each):

1) rabbits with high sympathetic tone – Mo ≤ 1.25 s; AMo ≥ 21%; MxDMn ≤ 0.10 s;

2) rabbits with high parasympathetic tone – Mo ≥ 1.41 s; AMo ≤ 15%; MxDMn ≥ 0.36 s;

3) rabbits with balanced autonomic tone – Mo 1.26–1.40 s; AMo 16–20%; MxDMn 0.11–0.35 s.

All procedures complied with ethical standards for animal experimentation (Directive 2010/63/EU, 2010). Animals were euthanized by inhalational overdose of chloroform.

Fragments from the thoracic esophagus were excised and fixed in Bouin's solution, dehydrated in graded ethanol (70–96%), cleared in xylene, and embedded in paraffin. Sections of 7μm thickness were cut, dried, deparaffinized (xylene), rehydrated in descending ethanol concentrations (96–70%), stained, and mounted under coverslips (Mulisch & Welsch, 2015). Esophageal wall structure was examined in sections stained with: 1) Mayer's hematoxylin and eosin; 2) aldehyde fuchsin (Gabe–Dyban method). Collagen fibers were demonstrated using Heidenhain's Azan stain. Histochemical reactions were also performed to detect neutral mucosubstances (Periodic Acid Schiff, PAS) and acidic mucosubstances (Alcian blue, pH 2.5 [AB]).

Morphometric analysis and photomicrography were performed using a Leica DM2500 light microscope equipped with a Leica DFC450C camera and Leica Application Suite 4.4 software (Leica Microsystems GmbH, Germany). On histological sections, the areas of the entire esophageal wall and its layers were measured using Aperio ImageScope software (Leica Microsystems GmbH, Germany, 2012). Measurements were expressed in absolute (mm²) and relative (%) values.

Statistical analysis was performed using one-way analysis of variance (ANOVA) with Bonferroni correction. The following parameters were calculated: sample mean (x), standard error (SE), and coefficient of variation (CV). Differences were considered significant at $P < 0.05$. Calculations were performed using StatPlus software (AnalystSoft Inc., USA, 2008). Relationships between variables were assessed using Pearson's correlation coefficient within each group.

Results

Morphometric analysis demonstrated a relationship between autonomic regulation type and esophageal wall morphology in the rabbits (Table 1). The rabbits with balanced autonomic tone exhibited the highest variability for most parameters; in some cases, their coefficients of variation were two- to threefold higher than in the other groups. The lowest variability was observed in the rabbits with high parasympathetic tone.

When characterizing the esophageal wall, it is evident that nearly two-thirds of its area (Table 2) was occupied by the muscular membrane (62.06% in the rabbits with high sympathetic tone, 61.10% in the rabbits with balanced autonomic tone, and 67.34% in the rabbits with high parasympathetic tone). In the muscular membrane as a whole and in all its layers (outer longitudinal, circular, and inner longitudinal; Fig. 1), the same relationship with autonomic regulation type was observed: We observed the largest areas in the rabbits with high parasympathetic tone, average areas in the rabbits with high sympathetic tone, and the smallest in the rabbits with balanced autonomic tone. The maximal muscular membrane area exceeded the minimal value by 1.465 mm² ($P = 0.0430$).

The muscular membrane, both as a whole and in its individual layers, also showed numerous correlations with other structural components of the esophageal wall.

Table 1
Area of structural components of the rabbit esophagus (x ± SE, n = 5)

Indicator	Animal group		
	rabbits with high sympathetic tone	rabbits with balanced autonomic tone	rabbits with high parasympathetic tone
Esophagus cross-sectional area, mm²	16.391 ± 0.718	15.076 ± 1.430	15.973 ± 0.384
Lumen area, mm²	1.321 ± 0.279	1.146 ± 0.183	1.156 ± 0.118
Wall area, mm²	15.070 ± 0.518	13.930 ± 1.279	14.817 ± 0.337
Mucosa area, mm²	3.162 ± 0.099	3.027 ± 0.172	2.827 ± 0.055
– epithelium area, mm²	1.925 ± 0.050[b]	1.788 ± 0.134	1.704 ± 0.027[b]
– keratinized epithelial layer area, mm²	0.752 ± 0.039[c]	0.585 ± 0.055[c]	0.720 ± 0.042
– lamina propria area, mm²	0.550 ± 0.042	0.489 ± 0.056	0.462 ± 0.027
– muscularis mucosae area, mm²	0.687 ± 0.014	0.750 ± 0.022[a]	0.661 ± 0.018[a]
Submucosa area, mm²	1.904 ± 0.101[bc]	1.423 ± 0.172[c]	1.509 ± 0.074[b]
Muscular membrane area, mm²	9.353 ± 0.461	8.512 ± 0.507[a]	9.977 ± 0.281[a]
– muscular membrane's inner longitudinal layer area, mm²	2.864 ± 0.143	2.654 ± 0.114[a]	3.131 ± 0.103[a]
– muscular membrane's circular layer area, mm²	4.897 ± 0.253	4.572 ± 0.769	5.162 ± 0.220
– muscular membrane's outer longitudinal layer area, mm²	1.592 ± 0.150	1.286 ± 0.091[a]	1.684 ± 0.052[a]
Serous membrane area, mm²	0.651 ± 0.067	0.968 ± 0.217	0.504 ± 0.079

Note: for each parameter, different letters indicate significant differences between animal groups according to Tukey's test ($P < 0.05$): [a] – between rabbits with balanced autonomic tone and rabbits with high parasympathetic tone; [b] – between rabbits with high sympathetic tone and rabbits with high parasympathetic tone; [c] – between rabbits with high sympathetic tone and rabbits with balanced autonomic tone.

Table 2
Proportion of individual layers and their components in the total esophageal wall area (x ± SE, n = 5)

Indicator	Animal group		
	rabbits with high sympathetic tone	rabbits with balanced autonomic tone	rabbits with high parasympathetic tone
Mucosa share, %	20.98 ± 0.53	21.73 ± 1,03[a]	19.08 ± 0,50[a]
– epithelium share, %	12.77 ± 0.62	12.84 ± 035[a]	11.50 ± 0.27[a]
– keratinized epithelial layer share, %	4.99 ± 0.17[c]	4.20 ± 0.15[c]	4.86 ± 0.23
– lamina propria share, %	3.65 ± 0.21[b]	3.51 ± 0.26	3.12 ± 0.17[b]
– muscularis mucosae share, %	4.56 ± 0.16[c]	5.38 ± 0.31[ac]	4.46 ± 0.15[a]
Submucosa share, %	12.64 ± 0.26[bc]	10.22 ± 0.30[c]	10.18 ± 0.61[b]
Muscular membrane share, %	62.06 ± 1.10[b]	61.10 ± 1.25[a]	67.34 ± 0.81[ab]
– muscular membrane's inner longitudinal layer share, %	19.00 ± 0.36[b]	19.05 ± 0.44[a]	21.13 ± 0.64[ab]
– muscular membrane's circular layer share, %	32.50 ± 0.42[b]	32.82 ± 0.70	34.84 ± 0.75[b]
– muscular membrane's outer longitudinal layer share, %	10.56 ± 0.60	9.23 ± 0.43[a]	11.37 ± 0.38[a]
Serous membrane share, %	4.32 ± 0.43	6.95 ± 0.52	3.40 ± 0.48

Note: see Table 1.

Fig. 1. Structure of the muscular membrane of the esophagus in a rabbit with high parasympathetic tone: *1* – muscular membrane; *2* – muscular membrane's inner longitudinal layer; *3* – muscular membrane's circular layer; *4* – muscular membrane's outer longitudinal layer (Heidenhain's Azan stain)

The circular layer of the muscular membrane exceeded the combined area of the two longitudinal layers. Its proportion within the esophageal wall area was 32.50% in the rabbits with high sympathetic tone, 32.82% in the rabbits with balanced autonomic tone, and 34.84% in the rabbits with high parasympathetic tone. In the rabbits with balanced autonomic tone, the minimal circular layer area (4.572 ± 0.769 mm²) was associated with moderate variability (CV = 13.3%). The other groups showed lower coefficients of variation and larger areas of this layer: 0.325 mm² and 0.590 mm² greater in the rabbits with high sympathetic tone and the rabbits with high parasympathetic tone, respecively.

The area of the inner longitudinal layer was markedly smaller than that of the circular layer. It constituted nearly one-third of the total muscular membrane area and approximately one-fifth of the esophageal wall (19.00% in the rabbits with high sympathetic tone, 19.05% in the rabbits with balanced autonomic tone, and 21.13% in the rabbits with high parasympathetic tone). Under all autonomic regulation types, the inner longitudinal layer exhibited high homogeneity; in the rabbits with balanced and high parasympathetic tone, the coefficient of variation was below 10%. Consequently, a significant difference between these groups was detected (0.477 mm², P = 0.0149). Smaller differences (0.267 mm²) were observed between the rabbits with high parasympathetic and those with high sympathetic tone.

The outer longitudinal layer was approximately half the size of the inner longitudinal layer and accounted for about one-tenth of the esophageal wall area (10.56% in the rabbits with high sympathetic tone, 9.23% in the rabbits with balanced autonomic tone, and 11.37% in the rabbits with high parasympathetic tone). Unlike the previous parameter, the variability of area in the rabbits with balanced and high sympathetic tone increased substantially, reaching a moderate level (CV = 15.8% and 21.1%, respectively). A significant difference in the area of the outer longitudinal layer was found between the rabbits with high parasympathetic tone and those with balanced autonomic tone (0.398 mm², P = 0.0051), whereas the rabbits with high sympathetic tone differed from the parasympathetic group by only 0.092 mm².

In the rabbits with high sympathetic tone, sympathetic predominance was associated with a positive correlation between the areas of the circular and outer longitudinal layers (r = 0.919, P = 0.0272). In the rabbits with balanced (r = 0.904, P = 0.0354) and high parasympathetic tone (r = 0.935, P = 0.0193), a similar correlation was observed between the inner and outer longitudinal layers.

The mucosa (Fig. 2) formed longitudinal folds of varying height, characteristic of the esophagus. Because the area of the entire mucosa rather than regional thickness was measured, the presence of folds did not affect the results. Overall, the mucosa was markedly smaller than the muscular membrane and accounted on average for about one-fifth of the area of esophageal wall (20.98% in the rabbits with high sympathetic tone, 21.73% in the rabbits with balanced autonomic tone, and 19.08% in the rabbits with high parasympathetic tone). In contrast to the muscular membrane, higher values of most mucosa parameters were associated with the rabbits exhibiting pronounced sympathetic tone. In this group, the total mucosa area (3.162 ± 0.099 mm²) exceeded that in the rabbits with balanced autonomic tone by 0.135 mm² and that in rabbits with high parasympathetic tone by 0.335 mm². Only in the rabbits with balanced autonomic tone did total mucosa area show a positive correlation with the esophageal lumen area (r = 0.889, P = 0.0435). At the same time, under all autonomic regulation types, no significant correlations were found among individual structural components within the mucosa itself, although such correlations were present between the mucosa and other layers of the esophageal wall.

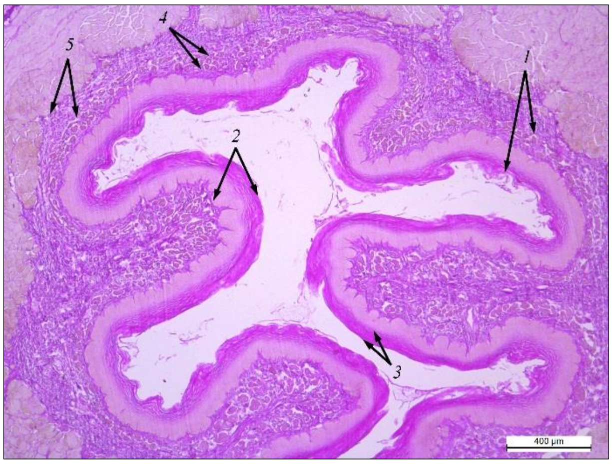

Fig. 2. Mucosa and submucosa of the esophagus in a rabbit with balanced autonomic tone: *1* – mucosa; *2* – epithelium; *3* – keratinized epithelial layer; *4* – muscularis mucosae; *5* – submucosa (PAS reaction)

More than half of the mucosa area was represented by the epithelium, which accounted for 12.77% of the total area of esophageal wall in the rabbits with high sympathetic tone, 12.84% in the rabbits with balanced autonomic tone, and 11.50% in the rabbits with high parasympathetic tone. The rabbits with high sympathetic and high parasympathetic tone showed high homogeneity of values and a significant difference between groups, with the former exceeding the latter by 0.221 mm² (P = 0.0076). In the rabbits with balanced autonomic tone, the coefficient of variation increased to a moderate level, and the epithelial area was 0.137 mm² smaller than in the rabbits with high sympathetic tone. A distinctive feature in the rabbits with balanced autonomic tone was a negative correlation between the epithelial area and the area of the inner longitudinal layer of the muscular membrane (r = −0.973, P = 0.0115).

Particular attention was drawn to the keratinized epithelial layer, which showed the highest concentration of neutral mucosubstances (Fig. 3A) and was the only structure containing acidic mucosubstances (Fig. 3B). By detecting these compounds, we were able to clearly delineate this layer and conduct an accurate morphometric assessment. According to the results, the area of the keratinized layer showed increased variability to a moderate level in all groups. The rabbits with balanced autonomic tone had the smallest area of keratinized layer (0.585 ± 0.055 mm²), being 0.167 mm² smaller than in the rabbits with high sympathetic tone (P = 0.0487) and 0.135 mm² smaller than in the rabbits with high parasympathetic tone. Marked intergroup differences were also observed in the proportion of the keratinized layer within the esophageal wall (4.99% in the rabbits with high sympathetic tone, 4.20% in the rabbits with balanced autonomic tone, and 4.86% in the rabbits with high parasympathetic tone).

The lamina propria was the smallest component of the mucosa. Its proportion within the total esophageal wall area was highest in the rabbits with high sympathetic tone (3.65%), intermediate in the rabbits with balanced autonomic tone (3.51%), and lowest in the rabbits with high parasympathetic tone (3.12%, Fig. 4). Unlike the epithelium, the lamina propria did not show a significant association with autonomic regulation type. This was due to minor intergroup differences and moderate (in the rabbits with high sympathetic and high parasympathetic tone) to high (in the rabbits with balanced autonomic tone) variability. Nevertheless, this parameter in the rabbits with high sympathetic tone exceeded that in the rabbits with balanced autonomic tone by 0.061 mm² and that in the rabbits with high parasympathetic tone by 0.088 mm².

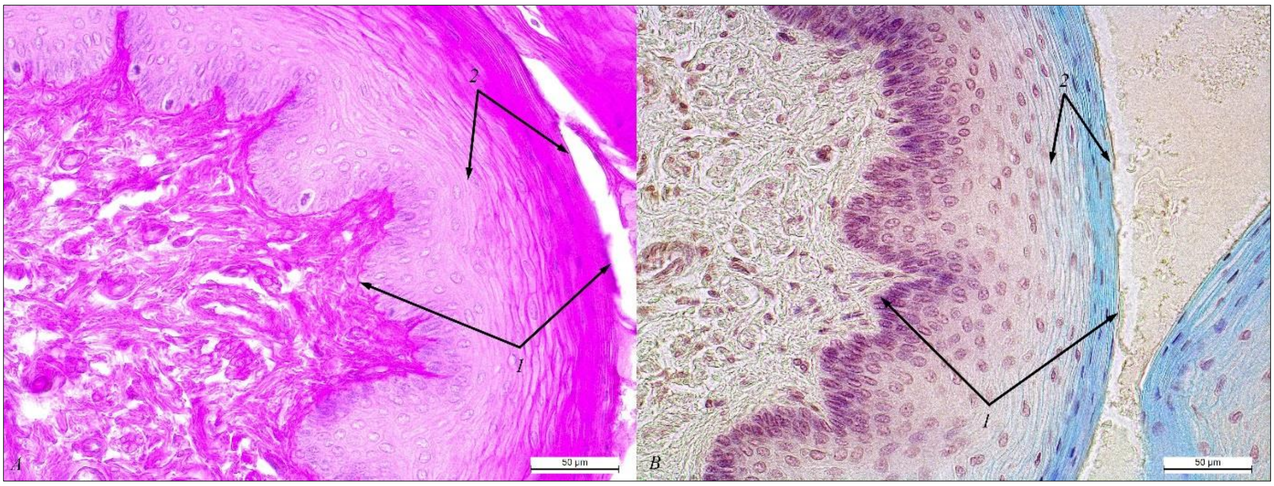

Fig. 3. Esophageal mucosa in a rabbit with high sympathetic tone: *A* – localization of neutral mucosubstances (PAS reaction); *B* – localization of acidic mucosubstances (Alcian blue reaction); *1* – epithelium; *2* – keratinized epithelial layer

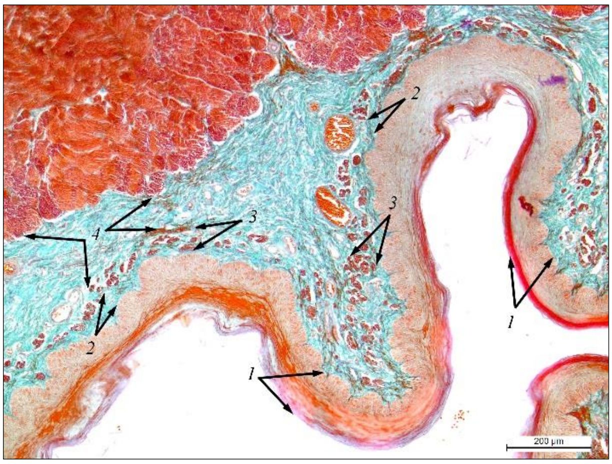

Fig. 4. Mucosa and submucosa of the esophagus in a rabbit with high parasympathetic tone: *1* – epithelium; *2* – lamina propria; *3* – muscularis mucosae; *4* – submucosa (aldehyde fuchsin staining by Gabe–Dyban)

A distinctive feature of the muscularis mucosae was the high homogeneity of its parameters, with the coefficient of variation below 10% in all groups. In contrast to other mucosa components, the largest area of the muscularis mucosae was observed in the rabbits with balanced autonomic tone, significantly exceeding that of the animals with high parasympathetic tone, with the difference between these groups measuring 0.089 mm² (P = 0.0151). The proportion of the muscularis mucosae within the esophageal wall structure was 4.56% in the rabbits with high sympathetic tone, 5.38% in the rabbits with balanced autonomic tone, and 4.46% in the rabbits with high parasympathetic tone. Under high parasympathetic tone, negative correlations were found between the area of the muscularis mucosae and the layers of the muscular membrane, particularly the inner longitudinal (r = −0.948, P = 0.0140) and outer longitudinal layers (r = −0.995, P = 0.0005). No such relationships were detected in the other groups.

The submucosa was connected to the mucosa with the muscular membrane and, on average, accounted for about one-tenth of the esophageal wall area (12.64% in the rabbits with high sympathetic tone, 10.22% in the rabbits with balanced autonomic tone, and 10.18% in the rabbits with high parasympathetic tone). Given the low variability of the parameters, a significant difference was established between the rabbits with high sympathetic tone and the rabbits with balanced autonomic tone (0.481 mm²; P = 0.0286), with predominance of the former group. The rabbits with high parasympathetic tone also had smaller values compared with the rabbits with high sympathetic tone (difference 0.396 mm²; P = 0.0395). In the rabbits with high sympathetic tone and the animals with balanced autonomic tone, the submucosa area was positively correlated with the total muscular membrane area (r = 0.987, P = 0.0019 and r = 0.988, P = 0.0022, respectively) and with the circular layer area (r = 0.887, P = 0.0441 and r = 0.982, P = 0.0028). Additionally, under high sympathetic tone, a positive correlation with the outer longitudinal layer area was observed (r = 0.974, P = 0.0050), whereas under balanced tone it was correlated with the inner longitudinal layer area (r = 0.917, P = 0.0282).

The serosa exhibited the greatest variability of area within the esophageal wall (Fig. 5). The coefficient of variation was high in all autonomic types and reached 50.2% in the rabbits with balanced autonomic tone, preventing statistically significant intergroup differences. Nevertheless, by mean value (0.968 ± 0.217 mm²), this group exceeded the rabbits with high sympathetic tone by almost one-third (0.651 ± 0.067 mm²) and exceeded the rabbits with high parasympathetic tone by nearly twofold (0.504 ± 0.079 mm²). Compared with other layers, the contribution of the serosa to the total esophageal wall area was small: 4.32% in the rabbits with high sympathetic tone, 6.95% in the rabbits with balanced autonomic tone, and 3.40% in the rabbits with high parasympathetic tone.

The combined areas of the mucosa, muscular membrane, and serosa constituted the total esophageal wall area, whose contribution to the overall cross-sectional area of the organ exceeded 90% (91.94% in the rabbits with high sympathetic tone, 92.40% in the rabbits with balanced autonomic tone, and 92.76% in the rabbits with high parasympathetic tone). The esophageal wall area showed no significant association with autonomic regulation type, as high homogeneity in the rabbits with high sympathetic tone and the rabbits with high parasympathetic tone coincided with a small difference in the mean values (0.253 mm²). Although this parameter in the rabbits with high sympathetic tone was 1.140 mm² lower compared with the rabbits with balanced autonomic tone, the group displayed an increased variability (CV = 20.5%).

The shape of the esophageal lumen showed marked individual variation due to mucosal folds. The lumen accounted for less than 10% of the total cross-sectional area of the organ (8.06% in the rabbits with high sympathetic tone, 7.60% in the rabbits with balanced autonomic tone, and 7.24% in the rabbits with high parasympathetic tone). In all groups, the lumen area varied considerably, with the coefficient of variation reaching 47.3% in the rabbits with high sympathetic tone. This group also had the largest mean lumen area (1.321 ± 0.279 mm²), 0.175 mm² and 0.165 mm² higher than the rabbits with balanced autonomic tone and the rabbits with high parasympathetic tone, respectively.

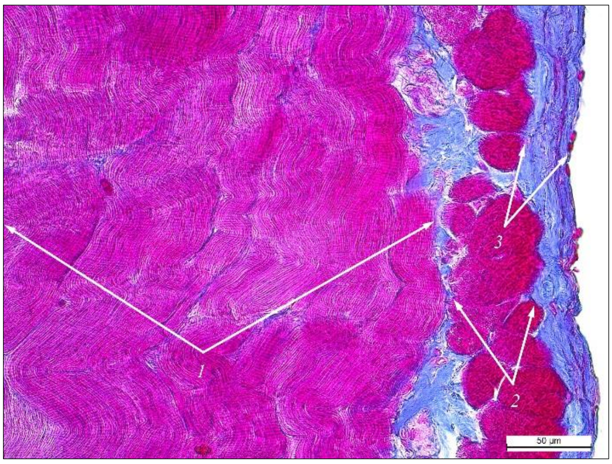

Fig. 5. Muscular membrane and serosa of the esophagus in a rabbit with balanced autonomic tone: *1* – circular layer of the muscular membrane; *2* – outer longitudinal layer of the muscular membrane; *3* – serosa (Heidenhain's Azan staining)

Overall, the above parameters jointly determined the esophageal cross-sectional area. Autonomic regulation type had no significant effect on this parameter, although the highest value was observed in the rabbits with high sympathetic tone (16.391 ± 0.718 mm²). In the rabbits with high parasympathetic tone and balanced autonomic tone, the parameter was lower by 0.418 mm² and 1.315 mm², respectively. In addition, a high variability (CV = 21.2%) was observed in the group with balanced autonomic tone.

Discussion

The relationship between the sympathetic and parasympathetic divisions of the autonomic nervous system is complex, encompassing both linear and nonlinear interactions, and cannot be described as a "zero-sum" system characterized by simple oscillations. Increased parasympathetic activity may be associated with a decrease, increase, or no change in sympathetic activity. Similarly, elevated sympathetic activity may occur independently of parasympathetic changes (Shaffer & Ginsberg, 2017). The resulting cumulative autonomic tone determines the level of regulatory influence on the organism.

The type of autonomic regulation was manifested not only in the absolute values of the measured parameters but also in their deviation from the mean. Pronounced predominance of sympathetic tone or parasympathetic tone positively influenced the parameter's homogeneity, with the coefficients of variation more often low and less frequently moderate. This suggests the formation of a stable morphofunctional profile of the esophagus under these autonomic regulation types. The increase in the parameter's variability in the rabbits with balanced autonomic tone indicates that a combination of sympathetic and parasympathetic influences may involve substantial individual variability within this group.

Overall, the constitutive model of the esophagus, derived from its biomechanical analysis, demonstrates a complex internal morphology, geometry, and mechanical properties, which are based on fiber characteristics, their quantity, and spatial arrangement (Natali et al., 2009). Our results indicate that the type of autonomic regulation influences the formation of this model.

The morphometric analysis of the esophageal wall area in rabbits with different autonomic regulation types showed some differences compared with the measurements of the wall thickness (Tybinka & Zakrevska, 2025). Measuring the area provides a more objective assessment, encompassing the entire structure, whereas thickness is measured only in discrete sites, which introduces potential errors (especially in folded layers such as the mucous membrane).

The formation of mucosal folds in the esophagus, as in other tubular organs, has important functional significance (Liao et al., 2007) and is associated with biomechanical properties that generate residual stresses in the growing tissues (Li et al., 2011). During the passage of a food bolus, these stresses are transformed into axial deformation, varying along the esophagus (Lu & Gregersen, 2001). These processes are determined by the rounded esophageal shape and the mechanical properties of its layers, forming an inverse relationship between wall thickness and intraluminal pressure (Liao et al., 2006; Sokolis, 2010). Our data suggest that the stress–strain relationship depends on autonomic regulation type and influences esophageal function. Mechanical properties of the esophageal wall are also determined by the presence of collagen and elastic fibers and their spatial orientation in different layers. Accordingly, the mucosa and submucosa are stiffer than the muscular membrane in both longitudinal and circumferential directions (Stavropoulou et al., 2009; Sokolis, 2013). These relationships show topographical differences related to the morphology of individual muscular layers (Stavropoulou et al., 2012), which our results link to autonomic regulation type.

Studying the morphologic and physiologic characteristics of the esophagus in animals is important for human medicine, forming the basis for tissue engineering techniques used to develop esophageal implants (Sommer et al., 2013). Considering that humans also exhibit different autonomic regulation types, our findings have practical relevance.

The primary source of stress and deformation in the esophagus is its muscular membrane – a complex three-layered structure responsible for the contractile activity of the wall. The contractile properties of each layer depend on the orientation and quantity of fibers. The angle between circular and longitudinal fibers is critical for esophageal peristalsis, with a sharper angle facilitating greater shortening in the distal esophagus compared with the middle and proximal segments (Vegesna et al., 2012). The large area of the circular layer (relative to the longitudinal layers) indicates significant muscular effort required for its function: Contraction of the circular layer reduces lumen diameter, increasing pressure and enhancing local peristaltic waves (Brasseur et al., 2007). Our results show that increased parasympathetic tone most strongly promotes this activity.

The outer and inner longitudinal layers of muscular membrane play key roles in peristalsis. Due to their rich sensory innervation, they also provide information on the functional state of the esophageal wall (Mittal, 2013).

Since all muscular layers exhibit the same type of relationship with autonomic regulation (dominance in rabbits with high parasympathetic tone), it can be concluded that the autonomic nervous system uniformly influences the entire muscular membrane, which is essential for effective function. Normally, all three muscle layers demonstrate coordinated activity, manifesting as synchronous peristaltic contractions (Mittal et al., 2006). Asynchronous contractions, differing in timing or amplitude, indicate a peristaltic dysfunction, potentially causing dysphagia or esophageal pain (Jung et al., 2005; Mittal, 2016). Thickening of the distal muscular membrane may lead to regurgitation in rabbits (Parkinson et al., 2017). Therefore, measuring the area of the muscular membrane and its individual layers can have diagnostic value, accounting for autonomic regulation type to determine normal dimensions for each animal.

Based on the area of individual layers and the whole muscular membrane, we infer that high parasympathetic tone in rabbits ensures the highest level of metabolic and trophic processes in this area. Pronounced sympathetic tone in rabbits reduces these to intermediate levels, whereas minimal values in rabbits with balanced autonomic tone likely reflect the suppressive effect of sympathetic centers over parasympathetic stimulation. Further studies are needed to clarify this feature. Unlike the stomach and intestines, the esophagus does not perform digestive functions but serves a transport role. Nevertheless, its mucous membrane is exposed to significant mechanical (solid food particles) and chemical (reflux) influence (Becskeházi et al., 2020; Visaggi & Dellon, 2025). The epithelium is the most vulnerable component, and pre-epithelial, epithelial, and postepithelial protective mechanisms are established (Sarosiek & McCallum, 2000; Blevins et al., 2018), collectively known as "esophageal mucosa resistance" (Orlando, 1998; Bertin et al., 2025). Keratinization of the epithelium is a major manifestation of this protective system. The results of our research prove that in extreme variants of autonomic tone, whether sympathetic or parasympathetic, epithelial protection is nearly identical, with a slight advantage of the first type. Under balanced autonomic tone, the keratinized area is reduced, although this does not indicate poorer protection, as lower mechanical load may suffice.

The intrinsic contractile activity of the mucousa is partially mediated by its muscularis mucosae, which exhibits notable species-specific morphofunctional characteristics (Uchida & Kamikawa, 2007). In rabbits, the muscularis mucosae has simple excitatory innervation and weak response to pharmacological stimulation (Percy et al., 1997). We observed a significant correlation between the muscularis mucosae area and autonomic regulation type, distinct from that seen in the muscular membrane.

The serous membrane, due to its mechanical characteristics, maintains the integrity of the esophageal wall and its resistance to mechanical damage during functional loads and diagnostic procedures (Zhang et al., 2018; Bourhis et al., 2020). High variability of its area in the rabbits of all groups reflects pronounced morphological and functional differences.

Despite morphometric differences in individual wall layers across autonomic regulation types, the esophageal wall maintains a relative structural stability. This is evident in the relative proportions of its layers. The mucosa, submucosa, muscular membrane, and serous membrane accounted for 20.98%, 12.64%, 62.06%, and 4.32% in the rabbits with high sympathetic tone; 21.73%, 10.22%, 61.10%, and 6.95% in the rabbits with balanced autonomic tone; and 19.08%, 10.18%, 67.34%, and 3.40%, respectively, in the rabbits with high parasympathetic tone. These proportions indicate the structural integrity and balance of the esophageal wall, which adapts to influences of autonomic regulation.

Conclusion

The morphology of the thoracic part of the rabbit esophagus depends on the characteristics of autonomic tone in the organism. The type of autonomic regulation, which served as the basis for grouping the animals, is reflected in the area of individual structural components of the esophageal wall. No group, however, exhibited a complete predominance in all measured parameters. Sympathetic autonomic tone positively influences the development of a larger mucosa. The rabbits with high sympathetic tone had the greatest area of the entire mucosa, its epithelium, the keratinized layer, the lamina propria, and the submucosa. Intermediate and smallest values of these parameters did not follow such a clear pattern.

In the muscular membrane, the opposite pattern was observed. It was entirely under the stimulatory influence of parasympathetic tone, since the rabbits with high parasympathetic tone exhibited the largest areas of all three muscular layers. The rabbits with balanced autonomic tone exhibited intermediate values, while the rabbits with high sympathetic tone had the smallest areas. The muscularis mucosae deviates from this sequence, as its area was the largest in the rabbits with balanced autonomic tone and the smallest in the rabbits with high parasympathetic tone. Rabbits with balanced autonomic tone also dominated in the area of the serous membrane. At the level of the whole organ, the overall advantage belonged to the rabbits with high sympathetic tone, which had the largest total cross-sectional area of the esophagus, its lumen, and the entire wall.

Autonomic regulation type also influenced the variability of the measured parameters. Intermediate and high variability was found in the rabbits with balanced autonomic tone. A shift of autonomic balance toward high sympathetic tone and especially high parasympathetic tone reduced the coefficient of variation to low and intermediate levels. Among all esophageal wall structures, the serous membrane exhibited the highest heterogeneity.

The entire muscular membrane and its individual layers formed numerous correlations with other structural components of the esophageal wall.

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