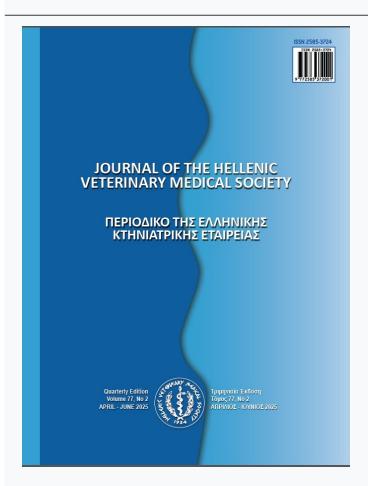




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# Morphometric Features of the Thoracic Esophagus in Rabbits

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**ABSTRACT:** The morphometric study of the esophagus wall was carried out on the cross-section of the thoracic part of the esophagus in male rabbits (Oryctolagus cuniculus). The area of individual membranes and their structural parts was determined, the correlation between them was established, and the features of their structure and shape were characterized. The superiority of area indicators over linear dimensions (thickness) morphometric studies was demonstrated. The quantitative and topographic characteristics of connective tissue in general and its fibers in particular were described. It was found that individual differences in the number of mucosal folds caused a high variability in the esophageal lumen area (CV=46.12%), which occupies 8% of the whole organ's cross-sectional area. These folds also determined the topographical features of the structure and size of the muscularis mucosae and the submucosa. Of the total area of the esophageal wall (14.64 mm<sup>2</sup>), the proportion of individual membranes was as follows: 20.8% mucosa, 11.2% submucosa, 62.6% muscular membrane, and 5.4% serous membrane. More than a half (60.4%) of the mucosal area was occupied by the epithelium, the keratinized layer of which was characterized by the highest concentration of neutral mucosubstances and was also the only site where acidic mucosubstances localization. Of the three muscle membrane layers, the circular layer occupied the largest area (4.81 mm<sup>2</sup>), the inner longitudinal layer reached average values (2.85 mm<sup>2</sup>), and the outer longitudinal layer demonstrated the smallest value (1.49 mm<sup>2</sup>). The indicators of the muscle membrane correlated with the area of the muscularis mucosae and the submucosa. The low and medium variability levels of most indicators showed the homogeneous structure of the esophageal wall. The area of the serous membrane was the most variable (CV = 58.20%) and had the highest density of collagen fibers. The concentration of elastic fibers peaked at the junction of the submucosa with the muscular membrane.

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**Keyword:** rabbit esophagus; mucosa; muscular membrane; serous membrane.

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### INTRODUCTION

Rabbits are often used as laboratory animals to study the functional arms. study the functional properties of the organism and the external influences on it (Mapara et al., 2012). In particular, the rabbit esophagus is most commonly used to study gastroesophageal reflux disease (Johnson and Harmon, 1986; De Hertogh et al., 2006). The processes that occur occurring in the esophagus under the influence of a stress factor (Tobey et al., 1999), cigarette smoke (Orlando et al., 1986) or alcohol (Bor and Capanoglu, 2009) are also considered. The studies are usually based on the analysis of changes in the structure of the esophageal wall. First of all, it is necessary to understand the normal anatomy of the organ in detail. The wall of the rabbit esophagus has a typical tubular structure. The mucosa forms folds that are lined with epithelium: non-keratinized in the cervical part and keratinized in the thoracic and abdominal parts. The mucosa also has a welldeveloped lamina propria and a muscularis mucosae (lamina muscularis). The esophageal glands are grouped in the form of lumps or bunches of grapes separated by layers of connective tissue. They are located in the submucosa of the cervical esophagus, partially penetrate the muscular membrane and secrete a mucous secretion (Hussein et al., 2017). The muscle membrane is formed by three muscle layers: the outer and inner layers are elongated, while the middle layer is circular. They are formed by striated muscle tissue and are surrounded by the adventitia in the cervical part and by the serosa in the thoracic and abdominal parts (Ranjan and Das, 2016; Selim et al., 2017). Along the esophagus, there is a progressive thickening of the muscularis mucosae, which consists of smooth muscle (Hughes, 1955). The circular layer of the muscular membrane is also significantly thickened (Cecio, 1976).

The wall of the rabbit's esophagus is permeated with a considerable number of somatic and autonomic nerve formations grouped into extramural and intramural plexuses with sensory and motor nerves (Cecio and Califano, 1967). The main part of the intramural nerve plexus is concentrated between the muscle membrane layers. It contains vagal and adrenergic nerve fibers, that extend into the submucosa and muscularis mucosae in addition to the muscular membrane (Nishimura and Takasu, 1969). Esophageal peristalsis is mainly controlled by the vagus nerve (Park and Conklin, 1999). In this process, there is a complex interaction between nerve centers and peripheral structures in regulating the

function of striated and smooth muscles in different parts of the organ (Goyal and Chaudhury, 2008; Nikaki et al., 2019).

Morphometric examinations of the rabbit esophageal wall usually involves determining the thickness of the individual membranes (Mohammad et al., 2020; Cipou et al., 2021). However, in the area of the folds, the mucosal thickness increases 2-3-fold, which has a negative effect on the objectivity of the measurements. This problem can be eliminated by measuring the area of the membranes and their structural parts (layers) and by determining the ratio between them. No studies on such indicators have been conducted in rabbits so far. Therefore, this approach became the target of research.

#### MATERIALS AND METHODS

Seven clinically healthy male rabbits (Oryctolagus cuniculus) of the Blanc de Termonde breed, aged four months and weighing 3.6-3.9 kilograms, were selected for the study. Such a number of experimental animals is consistent with similar morphological studies.

The research was conducted in accordance with the ethical standards for the use of animals in experiments (Directive 2010/63/EU, 2010). The research methodology was approved by the Ethics Committee of Stepan Gzhytskyi National University of Veterinary Medicine and Biotechnologies in Lviv (Protocol No. 18 of October 11, 2023). The animals were euthanized by inhalation of an overdose of chloroform.

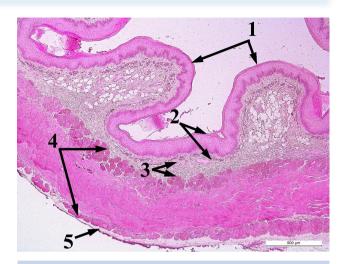
Fragments from the thoracic esophagus were isolated by transverse incisions, fixed in Bouin's solution for 24 hours, washed in 70% ethanol (24 hours), dehydrated in increasing concentrations (70-96%) of ethanol, clarified in xylene, and placed in paraffin blocks. From the latter, 7 µm thick sections were prepared and placed under a microscope slide after drying, deparaffinization in xylene, rehydration in decreasing concentrations of ethanol (96-70%), and staining (Mulisch and Welsch, 2015). The general morphology of the esophageal wall was examined on sections stained with: 1) Mayer's hematoxylin and eosin; 2) aldehyde-fuchsin of Gaba-Dyban. To detect collagen fibers, the sections were stained with azan according to the Heidenhain's method, and the elastic fibers with resorcinol-fuchsin according to the Weigert method. Histochemical reactions were also used to detect neutral mucosubstances (Periodic Acid Schiff - PAS) and acidic mucosubstances (Alcian blue, pH 2.5 (AB)).

Morphometric analysis and photography were performed using a Leica DM-2500 light microscope equipped with a Leica DFC450C camera and Leica Application Suite 4.4 software (Leica Microsystems GmbH, Germany). The area of the entire esophageal wall, its individual membranes and its structural components (layers) was measured absolutely (in mm²) and relatively (in %) on the histological specimens obtained. The morphometric computer programs ImageTool and WCIF ImageJ were used for this purpose.

Statistical analysis: the statistical processing of the research results was conducted using the Stat-Plus 2008 software. The following indicators were calculated: sample mean (M), standard error (SE), and coefficient of variation (CV). The relationship between individual parameters was determined using Pearson's correlation coefficient. The correlation was considered statistically significant at p<0.05.

#### RESULTS

According to the data in Table 1, the esophagus cross-sectional area (15.92 mm<sup>2</sup>) in the test animals was characterized by moderate homogeneity of the measurements (CV = 19.58%). The esophageal mucosa formed 2-5 large and 2-3 small folds in random order (Figure 1). The different number of folds in the animals caused considerable heterogeneity in the size of the esophageal lumen (CV = 46.12%), with its average area (1.28 mm<sup>2</sup>) representing only



**Figure 1.** The rabbit esophagus wall: 1 - mucosal folds; 2 - mucosa; 3 - submucosa; 4 - muscular membrane; 5 - serous membrane (Hematoxyline and eosin staining).

8% of the total cross-sectional area of the organ. Consequently, 92% of the area was occupied by the esophageal wall. The area of the esophageal wall was 14.64 mm<sup>2</sup>, with a coefficient of variation of 18.51%.

It is clearly noticeable that the mucosa is covered with keratinized epithelium, which has a uniform structure both on the folds and between them. The epithelium was the largest structural component of the mucosa, occupying 60.4% of its area. In the entire esophageal wall, the epithelium accounted for

Table 1. Area of morphological components of the rabbit of	esophageal wa	11	
Indicator	M	SE	CV (%)
Cross-sectional area, mm <sup>2</sup>	15.92	1.18	19.58
Lumen area, mm <sup>2</sup>	1.28	0.22	46.12
Wall area, mm <sup>2</sup>	14.64	1.02	18.51
Mucosa area, mm <sup>2</sup>	3.04	0.15	13.34
Epithelium area, mm <sup>2</sup>	1.84	0.10	14.03
Epithelium's keratinized layer area, mm <sup>2</sup>	0.67	0.07	27.91
Lamina propria area, mm²	0.51	0.05	24.65
Muscularis mucosae area, mm <sup>2</sup>	0.70	0.03	12.89
Submucosa area, mm <sup>2</sup>	1.64	0.17	27.66
Muscular membrane area, mm <sup>2</sup>	9.16	0.83	23.86
Muscular membrane's inner longitudinal layer area, mm <sup>2</sup>	2.85	0.19	17.29
Muscular membrane's circular layer area, mm <sup>2</sup>	4.81	0.58	31.76
Muscular membrane's outer longitudinal layer area, mm <sup>2</sup>	1.49	0.15	26.16
Serous membrane area, mm <sup>2</sup>	0.80	0.18	58.20

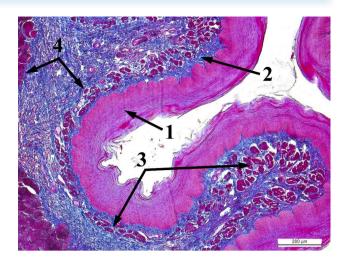
M - sample mean; SE - standard error; CV - coefficient of variation

12.6%. The absolute area of the epithelium (1.84 mm<sup>2</sup>) was characterized by relatively low variability (CV = 14.03%).

When examining the epithelium, it was assumed that the keratinized layer covers the entire surface from the point at which the first signs of keratinization appear (Figure 2). This is most clearly observed in histological sections after the PAS reaction. The keratinized layer of the epithelium showed the greatest heterogeneity in area measurements (CV = 27.91%) among all parts of the mucosa. The high variability in the measurements of the keratinized layer was obviously compensated by the minimal variability of the measurements of its non-keratinized portion, resulting in a low variability in the measurements of the whole epithelium. Overall, the area of the keratinized epithelial layer (0.67 mm<sup>2</sup>) constituted 36.4% of the total epithelial area, 22.1% of the mucosal area and 4.6% of the total esophageal wall area.

The distinctive feature of the epithelium's keratinized layer was that it was the only site in the rabbit esophageal wall where acidic mucosubstances were localized (Figure 2A). This area also had the highest concentration of neutral mucosubstances (Figure 2B).

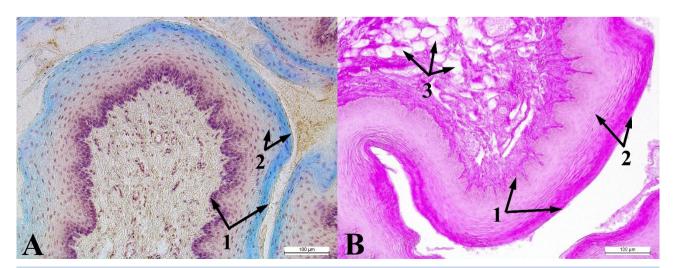
The lamina propria, consisting of connective tissue was the thinnest part of the mucosa. It formed numerous small protrusions that extended into the epithelium and, apparently, provided better adhesion between these layers (Figure 3). This was confirmed by a positive correlation (Table 2) between them (r = 0.942, p < 0.01). In the area of the mucosal folds,



**Figure 3.** Morphology of the mucous membrane and submucosa: 1 - epithelium; 2 - lamina propria; 3 - muscularis mucosae; 4 - submucosa (azan staining by Heidenhain).

the number and size of these protrusions were greater compared to the regions between the folds. The lamina propria area  $(0.51 \text{ mm}^2)$  exhibited a fairly high level of heterogeneity in measurements (CV = 27.91%). It accounted for 16.6% of the total mucosal area and 3.4% of the total esophageal wall area.

The muscularis mucosae was characterized by its fragmentary nature, as it did not consist of a continuous muscle strip but by bundles of myocytes of different thicknesses, separated by layers of connective tissue (Figure 3). The structure and dimensions of the muscularis mucosae were determined by its topography. In the intervals between the mucosal



**Figure 2.** A - localization of acidic mucosubstances (Alcian blue reaction); B - localization of neutral mucosubstances (PAS reaction). 1 - epithelium. 2 - keratinized layer of epithelium. 3 - deposition of adipose tissue in the submucosa.

Table 2. F	Table 2. Pearson's correlation coefficient	ıt							
Indicator	The means of the contract of T	Indicator number	ımber						
number	i ne name oi tne maicator	1	2	3	4	5	9	7	
	Epithelium area								
2	Lamina propria area	r = 0.9417 p = 0.0016							
33	Muscularis mucosae	r = 0.1914 p = 0.6809	r = 0.2323 p = 0.6161						
4	Submucosa area	r = 0.4904 p = 0.2638	r = 0.3825 p = 0.3971	r = -0.4825 p = 0.2728					
5	Muscular membrane's inner longitudinal layer area	r = 0.0067 p = 0.9887	r = -0.2037 p = 0.6613	r = -0.8401 p = 0.0180	r = 0.6148 p = 0.1418				
9	Muscular membrane's circular layer area	r = 0.5817 p = 0.1707	r = 0.4149 p = 0.3546	r = -0.4492 p = 0.3120	r = 0.7559 p = 0.0484	r = 0.7081 p = 0.0750			
7	Muscular membrane's outer longitudinal layer area	r = -0.1477 p = 0.7520	r = -0.2731 p = 0.5534	r = -0.6450 p = 0.1178	r = 0.6793 p = 0.0933	r = 0.7251 p = 0.0652	r = 0.5728 p = 0.1789		
∞	Serous membrane area	r = -0.2362 p = 0.6102	r = -0.025 p = 0.9576	r = -0,2330 p = 0,6151	r = 0.0156 p = 0.9735	r = -0.0395 p = 0.9330	r = 0,0441 p = 0,9252	r = 0.0251 p = 0.9574	

r - Pearson's correlation coefficient; p - level of statistical significance. The correlation was considered statistically significant at p<0.05.

folds, the muscle plate was either single-layered or double-layered. On the walls of the folds, it was mostly double-layered. Upon reaching the apex of the fold, the muscularis mucosae rarely remained the same and more often thickened becoming three-layered or four-layered (in broad folds) or even formed an oval thickening (in narrow folds). The presence of fatty tissue deposits in the submucosa had a negative effect on the structure of the muscle bundles. and reduced their thickness and number. However, no clear pattern for this process was established. Additionally, no connection was found between the number of the muscularis mucosae layers and the size and shape of the myocyte bundles that make it up. These features were characteristic of the esophagus in all examined animals, so the area of the muscularis mucosae (0.70 mm²) showed minimal variability in measurements (CV = 12.89%) not only within the mucosa but also in the entire organ wall. The muscularis mucosae accounted for 23.0% of the mucosal area and 4.8% of the esophageal wall area. A negative correlation was also found between the measurements of the muscularis mucosae and the internal longitudinal layer of the muscular membrane (r = -0.840, p < 0.05).

Combining the previous figures, we obtained the area of the total mucosa (3.04 mm<sup>2</sup>), which was quite homogeneous (CV=12.89%) and accounted for 20.8% of the total esophageal wall area.

The submucosa is mainly represented by connective tissue (Figure 3). It has invaded the inner longitudinal layer of the muscle membrane with wedge-shaped protrusions of different sizes, and has sometimes reached the circular layer (Figure 1). This prevents these membranes from separating during the movements of the esophageal wall.

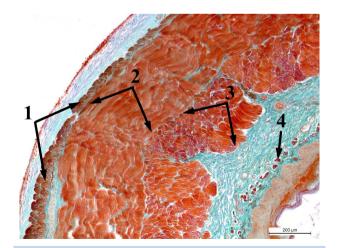
The submucosa thickness differed in various parts of the esophageal wall. Between the mucosal folds, its thickness was minimal, and in the folds' area it increased according to its size. These features had a lesser impact on the submucosa area. At the same time, the degree of its variability still remained quite high (CV = 27.66%), which is due to the different number of mucosal folds in individual animals. The average area of the submucosa ( $1.64 \text{ mm}^2$ ) amounted to 11.2% of the total organ wall and formed a positive correlation with the muscle membrane's circular layer area (r = 0.756, p < 0.05).

The submucosa contained accumulations of fatty tissue, that varied in size. Most of them were located in the area of the mucosal folds (Figure 2B).

The largest structure of the esophageal wall (both in terms of thickness and surface area) was the muscular membrane. Its area  $(9.16 \text{ mm}^2)$  accounted for 62.6% of the total wall area and was characterized by an average degree of variability (CV = 23.86%).

The muscle membrane is formed by three layers: the outer longitudinal, circumferential, and inner longitudinal one (Figure 4). Their thickness differed in various parts of the organ wall. With an increase in the thickness of one of the layers, either a corresponding thickening of the entire muscle membrane was observed, or its thickness remained almost unchanged. This indicates the formation of compensatory processes between the individual muscle layers, where an increase in the thickness of one layer was accompanied by a decrease in the thickness of another.

Differences in muscle fibers orientation determined the structural features of individual layers of the muscle membrane. In the transverse section of the esophageal wall, the circular layer looked like a homogeneous muscle plate with a minimal amount of connective tissue in the middle. In the thickness of both longitudinal layers, the amount of the connective tissue increased and divided them into bundles of different sizes. In this case, a larger fascicle could be formed as a result of the union of several small ones. In some places, it is noticeable that the strip of connective tissue partially divides the inner longitudinal layer into two smaller layers.



**Figure 4.** Muscular structures of the rabbit esophagus wall: 1 - muscular membrane's outer longitudinal layer; 2 - muscular membrane's circular layer; 3 - muscular membrane's inner longitudinal layer; 4 - muscularis mucosae (Gaba-Dyban aldehyde-fuchsine staining).

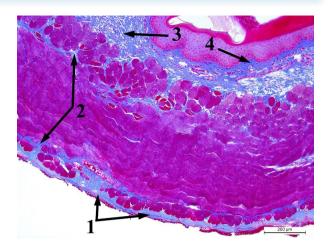
In the majority of esophageal wall sections (90%), the thickest part of the muscular membrane was the circular layer, which showed a rather high variability of area indicators (CV=31.76%) and its mean value was 4.81 mm<sup>2</sup>. At the same time, the size of this layer formed half (52.6%) of the area of the muscular membrane and a third (32.9%) of the total organ wall area.

The muscle membrane's inner longitudinal layer was characterized by smaller dimensions. However, in some esophageal wall areas (10%), it was thicker than the previous layer. The area of the inner longitudinal layer (2.85 mm²) had the highest homogeneity of parameters in the muscular membrane (CV=17.29%), occupying 31.1% of its size, as well as 19.5% of the total esophageal wall area.

A characteristic feature of the outer longitudinal layer of the muscular membrane was variations in shape, ranging from a plate of varying thickness to an oval thickening in adjacent areas of the esophageal wall. In general, the outer longitudinal layer was the smallest (1.49 mm²) in the muscular membrane, occupying only 16.3% of its area and 10.2% of the entire esophageal wall area. At the same time, the variability of its parameters was quite high, i.e., CV=26.16%.

Topographical features of the connection between the muscle membrane's individual layers were also revealed. In some places, neighboring layers were tightly adjacent to each other, which sometimes made it difficult to differentiate them. This was especially true in areas where the outer longitudinal layer had minimal thickness and was difficult to separate from the circular one. In other places, the muscle layers were separated by a strip of connective tissue of varying thickness.

In terms of size, however, the serous membrane was the most heterogeneous structure, with its thickness differing significantly on various surfaces of the esophagus (Figures 1, 5, 6). At the same time, the area with a thick serous membrane gradually or abruptly transitioned to an area with a minimal thickness, in which a layer of epithelial cells was attached to an almost unexpressed layer of connective tissue. Also, the serous membrane thickens in the areas of blood vessels, nerves, and adipose tissue accumulation. These features were combined in different ways in individual animals, which led to the highest variability of the serous membrane area (CV=58.20%) compared to other studied parameters. The contribution of the serous membrane area (0.80

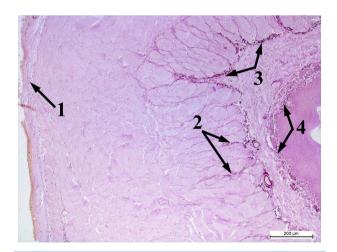


**Figure 5.** Localization of collagen fibers: 1 - in the serous membrane; 2 - between the layers of the muscular membrane; 3 - in the submucosa; 4 - in the lamina propria (azan staining by Heidenhain).

mm<sup>2</sup>) to the total dimensions of the esophageal wall was 5.4%. The basis of the serous membrane was formed by the connective tissue.

It is important to note that the serous membrane thickness had no significant correlation with any other studied index.

It has already been mentioned that the esophageal wall contained a significant amount of connective



**Figure 6.** Elastic fibers localization: 1 - in the serous membrane; 2 - in the muscular membrane's inner longitudinal layer; 3 - at the border between the submucosa and the muscular membrane; 4 - in the lamina propria and muscularis mucosae (Weigert's resorcinfuchsine staining).

tissue, the localization of which was not uniform in individual membranes. Also, the fibrous component, mainly formed by collagen fibers, was unevenly distributed in the connective tissue. Collagen fibers were most densely located in the serous membrane, where, due to the same circular spatial orientation, they often formed a homogeneous mass (Figure 5). In many places, it was stratified by the fatty inclusions, blood vessels, and nerves. In the muscle membrane, collagen fibers mostly form plates between individual layers and muscle fiber bundles. In the area of the circular layer, these plates were thinner and the collagen fibers were denser compared to the longitudinal layers.

In contrast to the muscle membrane, collagen fibers became the main structural part of the submucosa. Here, their arrangement was much looser and spatially multidirectional, due to the presence of mucosal folds. An increase in the amount of fatty deposits led to a decrease in the number of collagen fibers.

Different spatial orientations of collagen fibers was also characteristic of the muscularis mucosae. However, lamina propria was again characterized by a more ordered and therefore denser arrangement of these fibers.

Elastic fibers were quantitatively inferior to the collagen ones and demonstrated significant topographic features in the esophageal wall (Figure 6). In the serous membrane, their number was not substantial and almost all of them were dots or short curved lines. This indicated the cross-section of these longitudinally oriented fibers. The number of elastic fibers increased between the layers of the muscle membrane and the muscle fiber bundles of both longitudinal layers. Half of them already had the appearance of long wavy lines, indicating their circular orientation in the esophageal wall. In the middle of the circular layer, collagen fibers were single or formed small clusters. A similar situation was observed in the submucosa. However, in the area of its transition to the muscle membrane, the number of elastic fibers increased sharply, forming in many places a thin but well-defined plate that was immersed in the muscle membrane. The fibers had a longitudinal orientation. A large number of longitudinally oriented fibers were also found in the area of the lamina propria and muscularis mucosae.

## DISCUSSION

Determining the mucous membrane area and its layers made it possible to eliminate the problem of

folds and obtain more objective results compared to the linear dimensions (thickness) of the studied structures. The same also applies to the other two esophageal wall membranes (muscular and serous), which also have significant thickness fluctuations that are difficult to fully rely upon during linear measurement. Statistical analysis of the morphometry results revealed low and medium variability of most indicators, indicating the homogeneity of the esophageal wall structure and the complementarity of its parts.

Due to the small internal surface area of the mucous membrane and the short residence time of fluid in the esophagus, its epithelium does not play an important role in the transport of water and electrolytes. However, epithelial cells, like other cells, maintain homeostasis through transepithelial sodium transport, which allows maintaining the integrity and structure of the barrier between the lumen (external environment) and blood (internal environment) (Powell et al., 1975). Therefore, the study of the structural organization and morphometric parameters of the esophageal epithelial layer allows us to understand its role both in the normal functioning of the organ and the development of pathological conditions (Zhang et al., 2010). In particular, keratinization of the esophageal mucous membrane is an adaptive reaction that, on the one hand, prevents its mechanical damage by solid feed particles, and protects against chemical irritation during gastric acid reflux on the other hand. In clinical practice, this phenomenon is called 'esophageal mucosal resistance' (Orlando, 1998). Accordingly, the size of the keratinized layer of epithelium and its ratio to other layers of the mucous membrane indicate the level of esophageal wall protection. The presence of a correlation between the parameters of the lamina propria and the epithelium indicates their significant morphological and functional relationship.

The difference between the muscularis mucosae and the esophageal wall's muscle membrane is its fragmented structure. This is reflected in its functional properties and combined with pharmacological characteristics that differ from other parts of the rabbit digestive system (Uchida and Kamikawa, 2007). The muscularis mucosae undergo maximum contraction only under the influence of acetylcholine and are resistant to histamine, neurokinins, and prostaglandins, which have a strong excitatory effect on it in other parts of the intestine. The muscularis mucosae are closely related to the functioning of the mucosa in general and its epithelial layer in par-

ticular. At the same time, the relative simplicity of the muscularis mucosae structure indicates a lower functional activity of the esophageal mucosa compared to the following parts of the digestive system (Percy et al., 1997). The correlation with the muscle membrane characterizes the consistency of their contractile activity.

The division of the muscle membrane into separate layers determines different types of esophageal wall motor activity. The muscle membrane's longitudinal layers play a crucial role in the physiology of the sensory and motor functions of the esophagus. They are the basis of peristaltic contractions (Mittal, 2013). The circular layer creates pressure in the organ lumen during this process and also increases it, especially in the caudal part of the esophagus. The correlation found between this layer and the submucosa indicates the role of the latter in the process of muscle contractions.

The effective interaction between muscle layers is due to the different direction of their fibers (Vegesna et al., 2012). The close connection between the muscle membrane's longitudinal and circular layers is also indicated by their almost perfect synchronization during peristaltic contractions of a healthy esophagus (Mittal et al., 2006). Violation of this synchronization or separation of the muscle layers leads to symptoms such as dysphagia and esophageal pain.

Therefore, morphological and morphometric changes in one or all layers of the muscle membrane may be the signs of peristaltic dysfunction that occurs in the esophagus in certain diseases. For example, in eosinophilic esophagitis, pathological processes develop in the longitudinal layer of the muscle membrane (Jung et al., 2005; Mittal, 2016). Muscle membrane hypertrophy of the caudal esophagus may indicate the development of regurgitation (Parkinson et al., 2017). Thus, determining the area of the entire muscle membrane or its layers, as well as the ratio between them, can be used to determine the functional state of the organ and diagnose its diseases.

As a tubular organ, the esophagus is subjected to significant axial loads, which cause constant physiological fluctuations in its biomechanical and morphometric parameters. These processes are based on the features of the esophageal wall, which is formed by several layers of different types of tissues (Liao et al., 2006; Sokolis, 2010). The esophageal lumen area can characterize the level of axial deformation that occurs between its membranes (mucosa, mus-

cle, serosa) and is due to the different content of muscle and connective tissue elements in them (Lu and Gregersen, 2001).

The resistance of the esophageal wall to deformation largely depends on the content of elastin and collagen, the distribution of which differs in individual membranes. In the muscle membrane, the number of collagen and elastic fibers is lower than in the mucosa and submucosa, which makes these membranes more rigid. The majority of fibers are directed along the esophagus, which makes the esophageal wall stiffer in the longitudinal direction than in the transverse direction. In the submucosa and around the circular layer of the muscle membrane, some fibers are oriented in a circular pattern. The maximum saturation of connective tissue fibers is observed in the serous membrane of the esophagus, which makes it the most rigid structure of its wall (Stavropoulou et al., 2009; Sokolis, 2013). It is important to note that collagen fibers are mostly formed by type III collagen, while collagen types I, IV, and V are distributed in the form of amorphous substances in different layers of the esophagus (Schulze et al., 2001).

Since the number of the muscle membrane layers and their thickness vary along the esophagus, this is reflected in the saturation of the organ wall with collagen and elastic fibers. As a result, different parts of the esophagus (cervical, thoracic, and abdominal) differ in stiffness and biomechanical properties (Stavropoulou et al., 2012).

The heterogeneity of the serous membrane thickness on different surfaces of the esophagus may be due to the structure of the surrounding tissues. If the esophageal wall is adjacent to a significant layer of loose connective tissue, then its serous membrane is minimal in thickness. If the esophagus is in contact with skeletal muscles or the trachea, then its serous membrane thickens. The absence of a significant correlation between the serous membrane and the mucosa and muscle membranes indicates its insignificant role in ensuring the functional characteristics of the esophageal wall.

#### CONCLUSIONS

Determining the area of the esophageal wall's structures provided more objective results compared to their linear dimensions (thickness). When characterizing individual structures, it is important to know not only their absolute size but also the proportion they occupy in the total organ's area. The degree

of connective tissue development determines the morphological and morphometric features of all esophageal wall components, except for the epithelium. At the same time, adipose tissue deposition had a significant impact on the size and shape of both the entire esophageal wall and its parts. The esophageal wall is a homogeneous structure, as it is characterized by low and medium variability of most indicators. The correlation between individ-

ual layers indicates morphological and functional relationships among them. The established absolute and relative indicators of the esophageal wall will serve as a basis for comparison in the study of the functional characteristics and pathological processes of this organ.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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