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## **Table Of Contents**

Journal Cover	1
Author[s] Statement	3
Editorial Team	4
Article information	5
Check this article update (crossmark)	5
Check this article impact	5
Cite this article	5
Title page	6
Article Title	6
Author information	6
Abstract	6
Article content	7

Vol. 10 No. 2 (2025): December DOI: 10.21070/acopen.10.2025.12788

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Vol. 10 No. 2 (2025): December DOI: 10.21070/acopen.10.2025.12788

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Vol. 10 No. 2 (2025): December DOI: 10.21070/acopen.10.2025.12788

## Normal Macroscopic, Morphological and Morphometric Parameters of the Esophagus of White-Bred Rats

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#### **Abstract**

General Background: The esophagus plays a fundamental role in food transportation and peristaltic coordination within the digestive system, making it a vital subject of anatomical and physiological research. Specific Background: Despite its importance, there is limited quantitative information regarding the normal macroscopic and morphometric characteristics of the esophagus in laboratory rats, particularly in white-bred strains, which are essential for biomedical modeling. Knowledge Gap: Previous studies have focused primarily on pathological changes or chemically induced esophageal alterations, leaving a lack of standardized reference data for healthy specimens. Aims: This study aimed to characterize the normal macroscopic, morphological, and morphometric features of the esophagus in healthy white-bred rats under controlled laboratory conditions. Results: The findings revealed that the esophagus is a non-keratinized stratified squamous structure with progressive thickening of the muscular layers toward the lower segment, indicating adaptive enhancement for peristaltic propulsion. Quantitative measurements provided consistent baseline values across epithelial, submucosal, and muscular layers. Novelty: The study provides one of the most comprehensive baseline datasets for normal esophageal morphology in white-bred rats, bridging a critical gap in comparative and experimental anatomy. Implications: These reference values can serve as essential benchmarks for future pathological, toxicological, and pharmacological studies, improving the precision of experimental models involving the esophagus.

#### Highlight:

- The study presents normal macroscopic and morphometric parameters of the oesophagus in white-bred rats.
- Findings highlight gradual thickening of muscular layers indicating adaptive peristaltic function.
- Results provide baseline data valuable for pathological, pharmacological, and anatomical research.

Keywords: Oesophagus, Morphology, Morphometry, Histology, White-Bred Rats

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#### Introduction

Oesophagus makes an important component of the digestive system. It is a muscular passage that facilitates transit of food in the pharynx to the stomach through concerted peristaltic muscle contractions. Its construction offers mechanical protection and technical versatility, guaranteed to ensure the flawless passage of food without using materials going back. The oesophagus of mammals and especially rodents has very specific morphological layers, which are in line with its physiological requirements. Knowledge of normal architecture and morphometric characteristics of this organ is fundamental in translating mechanisms of disease, in assessing the effects of pharmacological agents to treat the disease, and also in the development of experimental surgical models [1].

The oesophagus has a number of separate layers anatomically: the mucosa/submucosa and the muscularis/adventitia. The different layers have a certain physiological function, like protection, secretion propulsion. Biomagnetically, similar to the rule of structural adaptation, the muscle layers of the oesophagus get thickened towards the gastric area improving work of peristalsis and stabilizing the gastric entrance pressure. Functional morphology and comparative anatomy indicate theoretical frameworks in which suggestions are that such structural gradients are due to the amount of mechanical stress adaptation and tissue specialisation. Thus, the determination of these parameters in experimental models of the gastrointestinal tract under controlled conditions provides insight into the evolutionary and physiological principles of the gastrointestinal tract [2].

Although a broad spectrum of studies examining the oesophageal pathology in humans exists to date, little information on normal macroscopic and morphometric parameters in laboratory animals is available. The morphometric structure of the healthy tissue has usually been neglected in the past, and previous literature concerning Wistar and Sprague-Dawley rats suggests oesophagitis, gastro-oesophageal reflux or chemically induced traumas. Moreover, by change of strain, diet and experimental design, the use of different levels of reference has led to inconsistency in reference values across different studies. Such gap in knowledge makes the interpretation of the pathological changes difficult and renders such comparative experiment inaccurate. Thus, systematic morphometric assessment of the normal oesophagus in white-bred rats should be conducted in order to provide credible basis to make anatomical and physiological comparison [3].

Under controlled environmental conditions, twelve, clinically healthy adult white-bred rats, were investigated in this study. Oesophagus was thoroughly dissected, sectioned and examined by applying histological staining and morphometry. Quantitative measurements were conducted to calculate the thickness of mucosal, submucosal and muscular layer whereas a qualitative one registered structural integrity and the organisation of the tissue. Mostly sophisticated imaging and statistical analysis were also used so as to create precision and reproducibility. Such methodological rigour sets apart the current study available information with previous reports on the descriptions which make it possible to have a well-rounded reflection of the variations of the segmentation according to the oesophageal wall [4].

It is believed that the results will offer the needed baselines that would be used in future experimental anatomical, pathological and pharmacological study. This paper provides a foundation to the determination of pathological changes in oesophageal diseases and the structure rearrangement of drugs by the determination of normal morphological and morphometric parameters. Also, the results can be extended in their comprehension of the biomechanical properties of peristalsis and tissue adaptation in humankind. It is the end result of this study that puts the classical essence of histology description and the modern day morphometry analysis back on its respective path, thus contributing towards the paramount body of theory and practice in veterinary and biological scientific disciplines [5].

#### Methods

In this study, 12 adult white-bred rats of both sexes aged between three and six months were used that were in sound clinical condition (weighing between 180 and 200 gm). The environment in which animals were kept had a regulated temperature relative humidity, and had a light/dark cycle of 12 hours. The rats were placed on a regular diet in the laboratory and allowed to drink water freely. The rats underwent acclimatisation of one week after which, more humanely isoflurane was administered to the rat and the ethical guidelines of the Bukhara State Medical Institute in accordance to European Directive 2010/63/EU. The thoracic and abdominal cavities were opened by a midline incision and the oesophagus was dissected meticulously on an entrance on the pharynx up to the cardiac esophageal opening with an intact structure being preserved. All specimens were rinsed with the use of an isotonic saline solution to cleanse the results and split into three parts, including upper (cervical), middle (thoracic), and lower (abdominal) so that they could be further analyzed [6].

The samples were 48 hours fixed in 10 natural buffered formalin, dehydrated in graded ethanol, cleared in xylene and embedded in paraffin wax. To analyze the general morphology, incrisions of 5 thick customary sections were performed on tissue using a Leica RM2235 rotary microtome and stained to view them with haematoxylin eosin because of the general morphology and the Masson trichrome to identify its regions i.e muscle and connective tissue segments. A light microscope, Leica DM500, was equipped with a Canon digital camera used to conduct microscopic analysis. ImageJ software was used to obtain morphometric measurements (thickness of the epithelial, submucosal and muscular layers) in each segment using ten randomly selected fields aided by ImageJ software. The outputs were in form of the mean standard error and were statistically calculated through GraphPad Prism programme. The significance was calculated by using one-way ANOVA then followed by post hoc test Using Tukleys post hoc test, the difference between the means would be considered statistically significant. Measurements were done twice by over data-grile analysts who verified that the analysis was precise and reproducible [7].

Vol. 10 No. 2 (2025): December DOI: 10.21070/acopen.10.2025.12788

#### **Results and Discussion**

Examining the mast of white rat revealed that their oesophagus always consisted of a straight tubing joining the pharynx with the stomach. It was averagely 4.2 0.3 long. The thickness of the walls was gradually increased to the lower part denoting slow muscular growth towards the gastric junction. (Table 1).

#### [ Table 1. is here ]

Histological analysis put forward four resolution layers which included mucosa, submucosa, muscularis and adventitia. There was non-keratinised stratified squamous epithelium in the mucosa in three zones: basal, intermediate and superficial that give mechanical protection. Lamina propria was loose connective tissue which was highly vascularized and had scattered immune cells [8].

The muscularis mucossae was of very fine fibres of smooth muscle which were more massive at whole thicknessen in the terminal part. Blood vessels, collagen fibres and mucous glands were present in submucosa. The muscularis propria was inner circular along with outer longitudinal layers of smooth muscle that were evidently divided by a plane of connective tissue. It is important to note that the thickness of muscular layer got increased considerably on the stomach (Table 2).

#### [ Table 2. is here ]

Under a microscope and a microscope, of the oesophagus, the white rats exhibited a well ordered modular structure, which was anatomically regulated into upper, middle and lower both of which showed characteristic morphometric features. The total oesophagus length was found to be 4.2 0.3 cm long, and the thickness of the wall was increasing slowly to the lower third area. The external diameter improved a bit (2.8 0.2 mm 3.1 0.2 mm) whereas wall thickness varied between (0.58 0.06 mm 0.64 0.08 mm). These minimal changes are probably a indicator of the adaptive hypertrophy of muscle necessary to provide effective peristaltic nutrient movement, and gastric control [9].

The presence of four major tunics (mucosa, submucosa, integrin in tunica muscularis and tunica); were confirmed by histological analysis (the mucosa, the submucosa, the tunica muscularis and the adventitia). Non-keratinised stratified squamous epithelium lined the mucosa which produces a strong mechanical force resistant to mechanics stresses applied during swallowing. The lamina propria distal was filled with fine vascular network and connectives tissues whereas submucosa was filled with loose collagenous fibres mixed with sporadic mucous glands. Such smooth muscle bundles were observed in the muscularis layer, in the form of circular and longitudinal corpus, where thickening was statistically significant (p < 0.05) in the lower segment. This trend supports this hypothesis that oesophagus of white-bred rats contributes to higher level of contractile efficiency towards the end of the stomach entrance [10].

Compared to the previous discussions, the morphological features detected are similar with other past researches with the Wistar and Sprague-Dawley rats with slight deviation in the percentage of the muscular laminar layer and the numbers of the submucosal glands distribution. Such variations might be attributed to the genetic strain variation and to the dietary adaptation. The distinct stratification of the epithelial layers that is not paralleled by the process of keratinisation also depicts the Oesophagal physiological mechanism of non-abrasive, soft food, which is one of the features of laboratory diets [11].

Theoretically speaking, the findings can be used to build a better understanding of oesophageal biomechanics in small mammals. The observed specialisation in the form of a segment, is indicative of the integration of functional specialisation, which accords with the overriding mechanical adaptation principle in the evolutionary biology of alimentary tract structures. The analysis validates that muscular deepening of the caudal oesophagus works to advance peristaltic check up alongside sphincter tallness and capture food in only one way instead of the other [12].

In reality, these morphometric bottoms play crucial roles in the toxicologies analysis, pharmacological modelling as well as surgery experimentation. It is also known that the normal morphometric values of oesophageal layers will facilitate the formation of a high degree of awareness on how specific drugs, exposure to chemicals or dieting can create a pathological alteration in the normal structure. More so, there are the data that are proveable in the refinement of animal models to study oesophagitis, dysphagia or smooth muscle disorder [13].

Nevertheless, based on these states of knowledge, there are still large gaps in knowledge. As a case in point, there is scarcity of research findings on the molecular and ultra structural correlates of oesophageal adaptation, such as collagen subtype localization, neural and vascular remodelling. Future research ought to utilize molecular histochemical and immunohistochemical platforms, 3D microCT analysis and biomechanical models in order to explain the micro structural dynamics that underlie the existent morphmetric differences. Also, it may be useful to further compare the development in morphometric differences in different rodent species and age groups in order to establish whether these morphometric changes occur species-specifically, or are species-convergent functional changes [14].

To sum up, in the present study, necessary quantitative and qualitative data about the oesophagus in white-bred rats are given, with special consideration to the constancy and the adaptative variability. The study opens the doors of fresh potential in the area of research in the fields of experimental anatomy, veterinary histology, and biomedical science at large as it combines classical morphology with contemporary analysis [15].

Vol. 10 No. 2 (2025): December DOI: 10.21070/acopen.10.2025.12788

### **Conclusion**

This research characterises extensively the normal macroscopic, morphological and morphmetric values of the oesophagus in healthy white rats, which forms a pivotal anatomical basis based on biomedical and veterinary studies. The results showed that the oesophagus rat is a well cellularised tubular orifice that bear four major tunics which include the mucosa, the submucosa, the tunica muscularis and the adventitia. Wall thickness and muscle-building are increasing gradually with rising segment up to bottom. This non-keratinised stratified squamous epithelium and gradually acquired thickening of circular and longitudinal layers of muscles indicate some structural adaptations such that enhance peristalsis and avert reflux of the gastric junction. These quantitative baseline offers some fundamental reference statistics on the ability to determine pathological deviations, evaluation of drug induced structural variations and experimental design enhancement of experimental model of oesophagitis diseases. The values of the morphometry developed at this point will contribute to the cross-species comparisons and increase the accuracy of the toxicological and pharmacological studies. Nevertheless, insufficient knowledge about the molecular and ultrastructural factors of oesophageal adaptation also finds its reflection in the current research. Further studies need to adopt a more sophisticated methodology (i.e. immunohistochemistry, molecular profiling, biomechanical modelling and 3D microCT imaging) in order to investigate the cellular and functional pathways of structural variation.

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