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Ultrastructural Study of the Digestive System in the Common Kestrel (*Falco tinnunculus*)

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Abstract

THIS STUDY utilized transmission electron microscopy (TEM) to examine the ultrastructure of the digestive system in the common kestrel (Falco tinnunculus), specifically focusing on various sections including the esophagus, proventriculus, and both small and large intestines, in relation to dietary adaptations. It was noted that the esophageal and large intestine mucosa predominantly contained a single type of epithelial cell. The proventricular cells' apex was distinguished by secretory granules, while the epithelial cells' apical region featured densely arranged, elongated microvilli. The small intestine's mucosa displayed diversity in epithelial cell types, including columnar epithelial cells, light and dark goblet cells, and Paneth cells. Goblet cells in the small intestine showed a striated border made of microvilli, mirroring those of columnar absorption cells, particularly evident in mature goblet cells prior to disruption by mucous mass expansion. In conclusion: The intricate structure of the kestrel's digestive tract and the specialized cellular makeup of its mucosal linings are intricately adapted to their specific digestive roles.

Keywords: Oesophagus, proventriculus, intestine, common kestrel, TEM, Falco tinnunculus.

Introduction

The European kestrel, known scientifically as Falco tinnunculus (Linnaeus, 1758), is a raptor within the Falconiformes order, Falconidae family, and Falco genus. Its diet predominantly consists of small mammals like voles and mice [1]

Research on birds of prey, such as the common kestrel, has been sparse due to challenges in acquiring specimens. An exception to this is a study [2] that offered comprehensive morphological insights into the kestrel's tongue and the entrance to its larynx. While there are detailed morphological studies of the gastrointestinal systems across various bird species [3-7], information on the gastrointestinal tracts of raptors is notably limited, with the exception of a study on the Eurasian hobby's stomach [8].

Understanding a bird's nutritional ecology necessitates a thorough knowledge of its digestive tract's morphology [9]. Until now, there has been a noticeable gap in literature regarding the transmission electron microscopy (TEM) analysis of

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the common kestrel's alimentary tract. Hence, this study seeks to explore the alimentary tract of the common kestrel through TEM and compare these findings to those of avian species with diverse dietary habits and histological study about alimentary tract and structure

Material and Methods

Samples

Ten alimentary tracts from normal adult healthy common kestrels (*F. tinnunculus*) were collected immediately after the birds were caught by a local hunter beside the Mediterranean Sea in Damietta, Egypt. These samples collected from common kestrels were required to be without any alimentary tract injuries or abnormalities[10]. This study adhered to the ethical standards and guidelines for the treatment and use of experimental animals as approved by the Animal Welfare and Ethics Committee at the Faculty of Veterinary Medicine, Kafrelsheikh University, in compliance with Egyptian legislation under the approval number KFS-IACUC/131/2021.

Gross morphology examination

Three alimentary tracts from the examined bird were prepared for the description of the gross morphological appearance. Subsequently, the morphological features were captured using a digital camera (Cannon IXY 325, Japan). The anatomical terminology applied in this study followed the standards set by the Nomina Anatomica Avium (NAA) [11]

TEM

Specimens from the alimentary tract were immediately preserved in a 6% phosphate-buffered glutaraldehyde solution (pH 7.4) at 4°C for six hours, following protocols described by [12, 13]. Post-fixation, the tissues underwent several rinses in cold 0.1 M phosphate buffer, then dehydrated in ascending ethanol concentrations, and cleared in propylene oxide before embedding in an epoxy araldite mixture overnight [14]. Initial semi-thin sections (1 mm) were stained with toluidine blue. Ultrathin sections (60-100 nm) were prepared with a glass knife on an L.K.B. microtome, stained with uranyl acetate and lead citrate [14, 15], and analyzed under a Jeol transmission electron microscope at 100 kV at Alexandria University's Faculty of Science.[27].

Data Analysis

Data were gathered and analyzed using SPSS software (version 20), with one-way ANOVA and Duncan's multiple range tests applied to pinpoint significant differences among the means. A p-value below 0.05 was deemed indicative of statistical significance.

Results

Esophagus long and thin-walled tube (Fig. 1) with a number of longitudinal folds that allow for a highly distensible character, thereby permitting the swallowing of large food particles. The oesophagus extended from the oropharynx to the proventricular part of the stomach without crop. The oesophagus was split up into two clear parts, the cervical and thoracic parts (Fig. 1). The cervical part began in the midline dorsal oesophagus to the trachea and then deviated to the right side, while the thoracic part began at the thoracic inlet and then returned to its dorsal position at the level of the 2nd rib. There are many mucous glands that lubricate food particles to aid in their passage to the stomach, which is clear from the observation of several dense secretory granules (Fig. 2).

The stomach, depicted in Figure 1, is an expanded muscular pouch within the digestive tract, segmented into two unique sections: the forward (cranial) section, known as the proventriculus or glandular part, and the rear (caudal) section, referred to as the ventriculus or muscular part, commonly known as the gizzard part. The two chambers were not separated from each other by an isthmus or intermediate zone. The transmission electron microscopic examinations of the proventricular cells show that the basal part of the proventricular mucosal cells have numerous rough endoplasmic reticula, which were surrounded by numerous round mitochondria and an oval nucleus (Fig. 3 and 4A), while the apical part of the cell was characterized by the presence of numerous round secretory granules (Fig. 3 and 4B).

The small intestine, illustrated in Figure 1, extends as a lengthy tube and is segmented into three regions: the duodenum, jejunum, and ileum. Characteristically, the duodenum forms a U-shaped structure comprised of two loops - an upper descending loop and a lower ascending loop. The transmission electron microscopic examinations of the small intestine make clear that there are different types of cells, namely, intestinal epithelial cells, dark and light goblet cells and Paneth cells (Fig. 5A). The oval-shaped Paneth cells have several secretory granules in their cytoplasm and are surrounded by numerous mitochondria (Fig. 5B and 6). The apical part of the intestinal epithelial cells contained microvilli with junctional complexes (Fig. 7 and 8).

Discussion

Reviews of the alimentary tract morphology across bird species reveal a link between avian structure and ecological behavior, including diet, feeding habits, and nutritional modes [8, 16-18]. Birds show significant specialization in their digestive tracts, indicative of evolutionary adaptation [19].

Avian stomachs typically feature two main sections: the proventriculus (glandular) and the ventriculus (muscular, also known as the gizzard), seen across various bird species. The division between these chambers does not depend on food type [8, 20-24]. It has been noted [25] that avian stomachs can have a third, the pyloric part, though it is smaller in domestic birds. An isthmus area, located between the proventriculus and ventriculus, has been identified [6, 26-29], but was absent in some birds, including the cattle egret, ibis, and Eurasian hobby, as found in this study and in [8, 30, 31].

The crop is generally present in most bird species [4, 32-34], though it is often missing in carnivorous birds, as observed in this research and in [8]. Similarly, a lack of a caecum was noted both in this work and in [34, 35].

This investigation found several mitochondria and secretory granules in the alimentary tract samples, aligning with findings in the spur-thighed tortoise Testudo graeca, which also showed numerous mucous granules and mitochondria [36].

TEM analysis of the proventriculus revealed numerous rough endoplasmic reticula and round mitochondria surrounding an oval nucleus in the basal part of mucosal cells, while the apical part contained many secretory granules. This contrasts with findings that the basal plasmalemma's infoldings contain chief cells without secretory granules, differing from this study's results and those in [37, 38]. Reports on the chief cells of the spur-thighed tortoise highlighted similar mucous granules [36]. The proventriculus's glandular epithelium, composed of simple cuboidal exocrine cells, including oxyntico-peptic cells secreting hydrochloric acid and pepsinogen, was noted in various animals, showcasing adaptations to their feeding styles[39, 40].

The small intestine's TEM examinations revealed diverse cell types, such as epithelial cells and goblet cells, mirroring observations in [16, 18, 41]. Goblet cells, essential for mucus production, aid in digesta movement and protect the mucosal surface, corroborating findings by [42-44]. Paneth cells, first described as pyramidal [40] and noted here as oval with numerous secretory granules surrounded by mitochondria, align with descriptions in [45, 46].

The small intestinal epithelial cells' apical part, containing many microvilli and junctional complexes, was similar to observations in[16-18, 41], and matches reports on absorptive cells' microvilli [36].

The large intestine's TEM examinations showed epithelial cells with numerous microvilli, mitochondria, and multivesicular bodies, in line with observations by [41], while mucous granules and mitochondria were also noted in the apical cells, and the nucleus and rough endoplasmic reticula in the basal part, as reported in [36].

This study's findings on microvilli and junctional complexes in the apical part of intestinal epithelial cells highlight a morphological basis for macromolecule absorption, consistent with observations in the great horned owl [47].

Conflict of interest statement

The authors have disclosed that they do not hold any conflicts of interest related to the publication of this article.

Funding statement

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Ethical Approval

This study adhered to the ethical standards and guidelines for the treatment and use of experimental animals as approved by the Animal Welfare and Ethics Committee at the Faculty of Veterinary Medicine, Kafrelsheikh University, in compliance with Egyptian legislation under the approval number KFS-IACUC/131/2021.

Author`s contribution

M.M.A.A.,F.F.,R.K., designed the study plan, F.F., M.S.,drafted the manuscript, **E.K.K.,H.H.,**helped in conducting the research work, conducting data analysis, and assisted in the writing of the manuscript, **M.S.,M.Y.M.** provided technical help in writing the manuscript, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

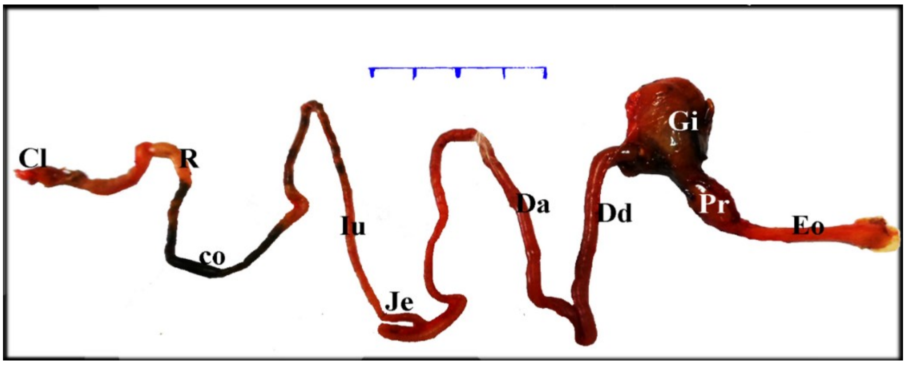

Fig. 1. Macrograph of the gastrointestinal tract of the common kestrel (*Falco tinnunculus*) showing oesophagus (Eo), proventriculus (Pr), gizzard (Gi), descending limb of the duodenum (Dd), ascending limb of the duodenum (Da), jejunum (Je), ilium (Iu), colon (co), rectum (R) and cloaca (CL).

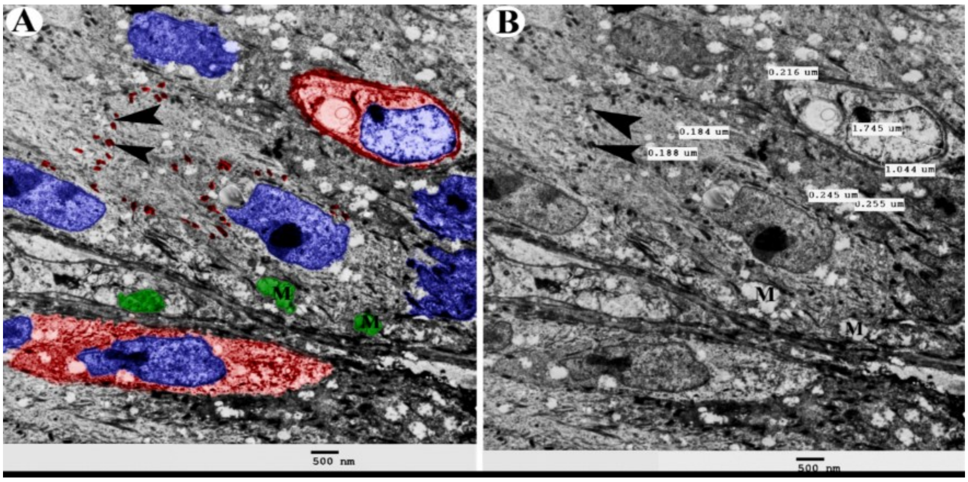

Fig. 2. Transmission electron micrograph of the oesophageal epithelial absorptive cell of the common kestrel (Falco tinnunculus) showing several mitochondria (M) and several dense secretory granules (black head arrows).

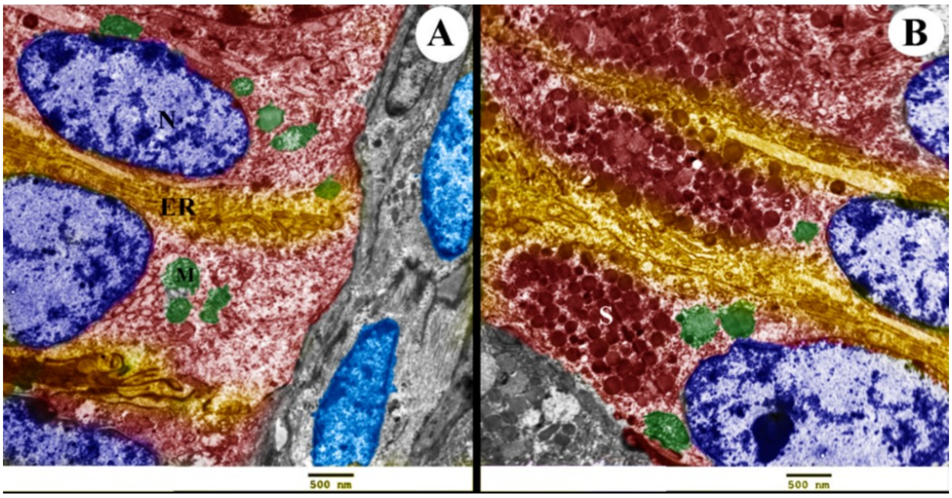

Fig. 3. Transmission electron micrograph of the proventricular mucosal cells of the common kestrel (*Falco tinnunculus*) showing numerous electron-

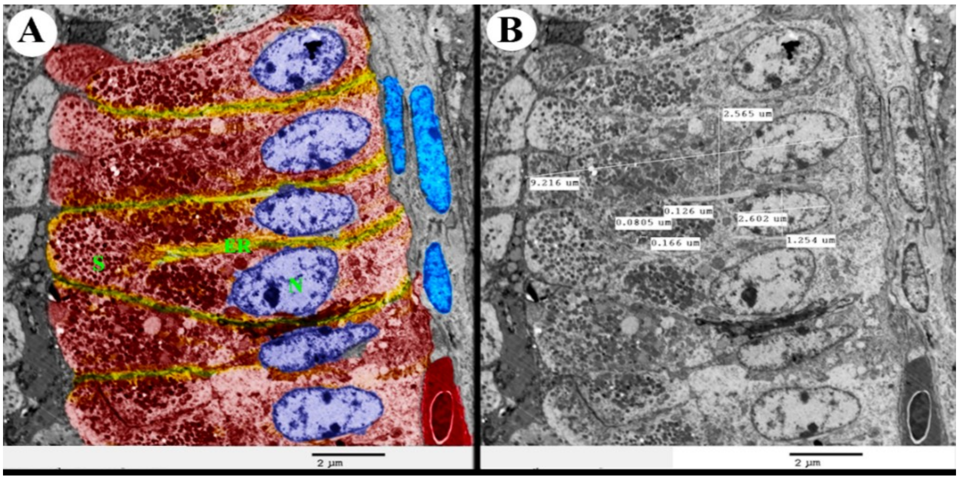

Fig.4. Transmission electron micrograph of the proventricular mucosal cells of the common kestrel (*Falco tinnunculus*):View (A), showing endoplasmic reticulum (ER) and the nucleus (N) in the basal part of the cell, and View (B), showing several secretory granules (S) in the apical part of the cell. dense secretory granules (S), nuclei (N) and endoplasmic reticula (ER).

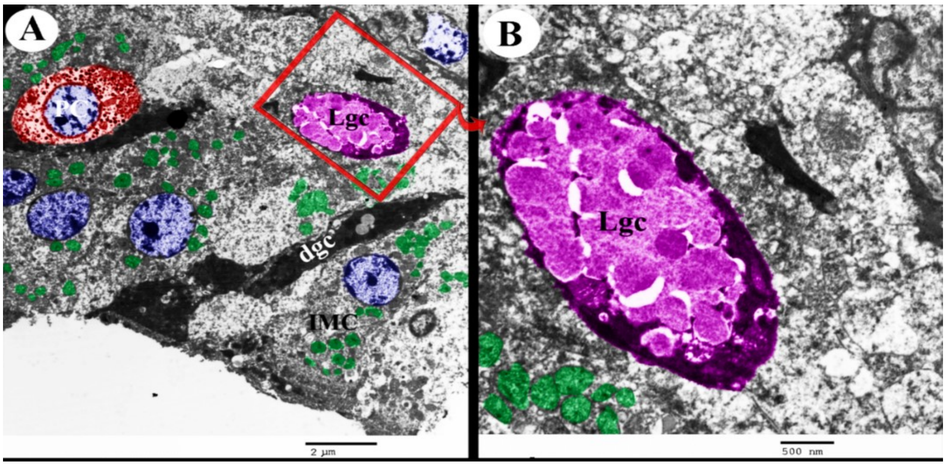

Fig. 5. Transmission electron micrograph of the small intestinal epithelial cells of the common kestrel (*Falco tinnunculus*) showing different types of cells, namely, intestinal mucosal cells (IMC), dark ((dgc) and light (Lgc) goblet cells and Paneth cells (PC).

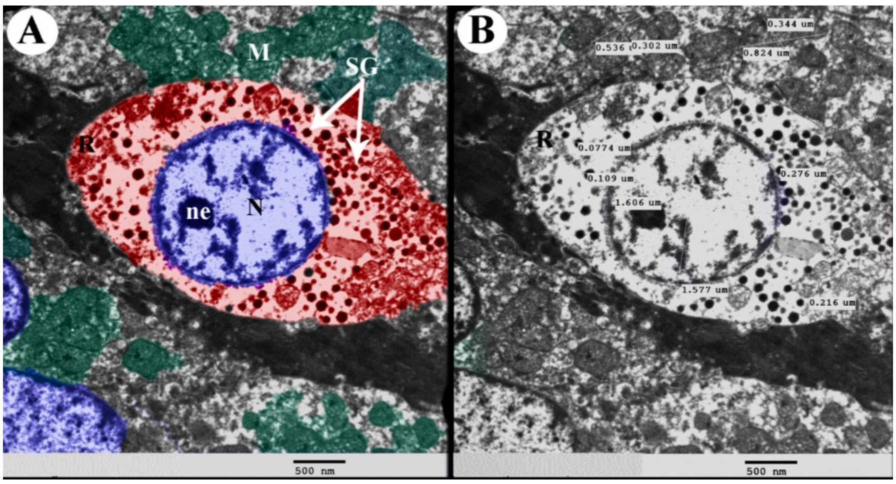

Fig. 6. Transmission electron micrograph of the small intestine of the common kestrel (*Falco tinnunculus*) showing an oval-shaped Paneth cell containing several secretory granules (SG) in the cytoplasm, a round nucleus (N) with its nucleolus (ne) and rough endoplasmic reticulum (R). The oval-shaped Paneth cell is surrounded by several mitochondria (M).

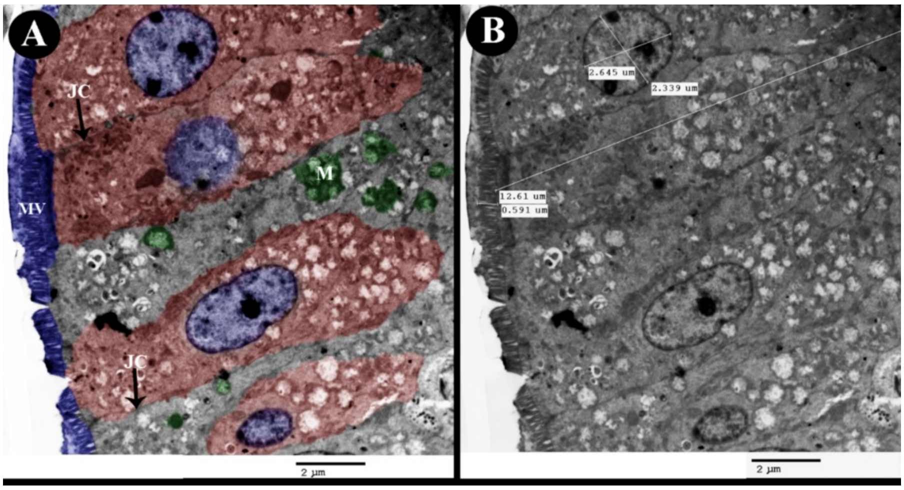

Fig.7. Transmission electron micrograph of the small intestinal epithelial cells of the common kestrel (*Falco tinnunculus*) showing microvilli (MV) with junctional complexes (JC) situated just below the free surface.

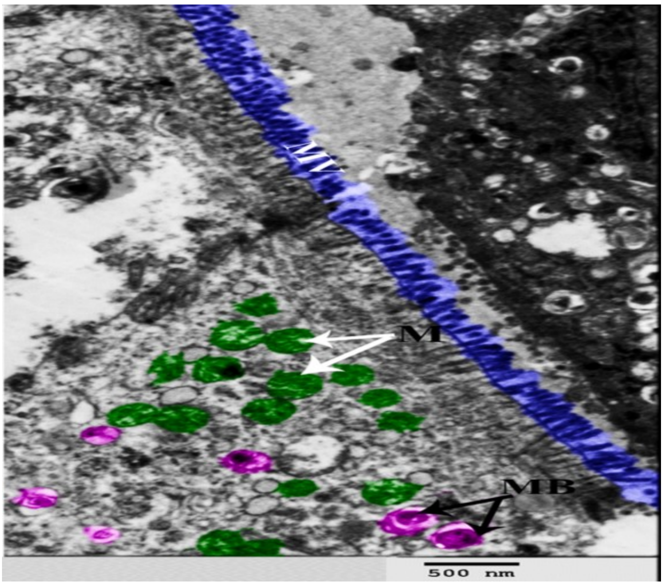

Fig. 8. Transmission electron micrograph of the large intestinal epithelial cells of the common kestrel (Falco tinnunculus) showing microvilli (MV), mitochondria (M) and multivesicular bodies (MB).

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دراسة التراكيب الدقيقة للجهاز الهضمي في الشاهين الشائع (Falco tinnunculus)

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[10] قسم علم الحيوان، كلية العلوم، جامعة أسوان، أسوان، مصر.المؤلف المراسل: مصطفى شكري، قسم الفسيولوجي، كلية الطب البيطري، جامعة كفر الشيخ، كفر الشيخ 33516، مصر.(mostafa.ataa@vet.kfs.edu.eg) .

المستخلص

استخدمت هذه الدراسة مجهر الإلكترون النافذ (TEM) لفحص التراكيب الدقيقة للجهاز الهضمي في الشاهين الشائع (Falco tinnunculus)، مع التركيز بشكل خاص على أجزاء متنوعة بما في ذلك المريء، والمعدة الغديّة، والأمعاء الصغيرة والكبيرة، وذلك في سياق التكيفات الغذائية. لوحظ أن مخاطية المريء والأمعاء الكبيرة تحتوي بشكل رئيسي على نوع واحد من الخلايا الظهارية. تميزت قمة خلايا المعدة الغديّة بحبيبات إفرازية، بينما تميزت المنطقة القمية للخلايا الظهارية بأهداب مجهرية مرتبة بكثافة وطويلة. أظهرت مخاطية الأمعاء الصغيرة تنوعاً في أنواع الخلايا الظهارية، بما في ذلك الخلايا الظهارية العمودية، وخلايا الغوبلت الفاتحة والداكنة، وخلايا بانيث. أظهرت خلايا الغوبلت في الأمعاء الصغيرة حداً مخططاً يتكون من أهداب مجهرية، تشبه تلك الموجودة في خلايا الامتصاص العمودية، وكان ذلك واضحاً بشكل خاص في خلايا الغوبلت الناضجة قبل تعطلها بسبب توسع كتلة المخاط. في الختام: تركيب الجهاز الهضمي المعقد للشاهين والتركيب الخلوي المتخصص لبطاناته المخاطية متكيف بدقة مع الأدوار الهضمية المحددة.

الكلمات المفتاحية: المريء، المعدة الغديّة، الأمعاء، الشاهين الشائع، مجهر الإلكترون النافذ، فلكو تينونكولوس.