



The histological and histochemical features of the esophagus in local breed dogs (*Canis familiaris*)

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Article information

Article history:

Received March 11, 2022

Accepted July 04, 2022

Available online July 04, 2022

Keywords:

Esophagus

Histological

Histochemical

Dog

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Abstract

A total of eight samples of the esophagus of local breed dogs are used to investigate the histochemical features of the esophagus. The specimens are processed according to paraffin embedding technique protocols and the tissue sections are stained with hematoxylin and eosin, and masons trichrome combined with Alcian blue (pH2.5)-PAS. The esophagus folds the mucosa which is covered by keratinized to non-keratinized stratified squamous epithelium which contains numerous excretory common ducts of esophageal glands. The epithelial thickness of cervical, thoracic and abdominal parts of the esophagus are $221.95 \pm 3.41 \mu\text{m}$, $212.46 \pm 5.38 \mu\text{m}$, and $173.15 \pm 4.09 \mu\text{m}$ respectively. The lamina propria of the cervical part of the esophagus lacks the muscularis mucosa, while the muscular mucosa in the thoracic part of the smooth muscles appears as scattered interrupted bundles. The esophageal glands are a type of compound tubular mucoserous constructed of the predominated mucous alveoli and little serous acini. When combined with Alcian blue (pH2.5)-PAS stain, the esophageal glands show strong acidic mucopolysaccharides within the mucous alveoli and light blue color within serous acini that denote weak acidic zymogen granules. Tunica muscularis is striated muscle fibers in cervical and thoracic parts and smooth in the abdominal part of the esophagus. The measured thickness of tunica muscularis of cervical, thoracic and abdominal parts are $568.76 \pm 6.90 \mu\text{m}$, $703.29 \pm 7.54 \mu\text{m}$, and $338.98 \pm 7.26 \mu\text{m}$ respectively.

DOI: [10.33899/ijvs.2022.133034.2164](https://doi.org/10.33899/ijvs.2022.133034.2164), ©Authors, 2022, College of Veterinary Medicine, University of Mosul.

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Introduction

Canis familiaris dogs are domesticated animals that belong to the ancient miss wolf which is characterized by an elevating tail. This dog species belongs to the genus *Canis*, derived from an old wolf (1,2). Many esophageal diseases can affect these dogs; stricture, vascular ring anomaly, and external body (3,4). Affected with megaesophagus is regarded as a prognosis for many disorders resulting in neuromuscular dysfunction (4). The esophageal disorder leads to many clinical symptoms such as weight loss, regurgitation, and chest pain. Esophageal achalasia results

from missing peristalsis that leads to the dilation of the organs, and at the same time, the cardia sphincter fails to respond normally to swallowing (5). The esophagus is a connection tube between the oral cavity and the stomach that passes the food easily. It is a hollow organ composed of four tunicates: mucosa, submucosa, muscularis externa, and adventitia (6,7). The cytoarchitecture of the esophagus differs according to various animal species, types of food, and behaviors. Numerous studies describe the morphological and histological structure of the esophagus in different animal species such as (6,7) in hummed camels, (8) in lamas (*Lama glama*), (9) in dogs, and (10) in a comparative study

of the histological structure of the esophagus in different mammals. The current study aims to investigate the main features of the esophagus in local breed dogs.

Materials and methods

Ethical approval

The design of the present study was approved by the Animal Care and Use Committee at the College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

Animal preparation

A total of eight samples of the esophagus of adult dogs were used for this study at the Department of Anatomy at the College of the Veterinary Medicine / University of Baghdad. The samples of the esophagus were obtained from many surgical hopeless cases of accidents from the Department of Surgery. The esophagus was removed from the cadaver, the tissue specimens were taken from cervical, thoracic and abdominal parts, and the esophagus was washed up with a normal saline solution (0.9%) and fixed by 10% buffered formalin saline for 48 hours. The tissue specimens were processed according to the paraffin embedding technique protocol (58-60°C) and the tissue was sectioned at 5-6µm by rotary microtome. The tissue sections were stained with Hematoxylin and Eosin, Masson's trichrome, and combined with Alcian blue (2.5pH) and PAS stain (11,12). The tissue sections were examined and photographed by Olympus microscope SC 35 camera. The thickness of the tunica of each esophageal part was analyzed and scored using the Fiji image analyzer system (13).

Results

The results reveal that the esophagus in local breed dogs is composed of four tunicates: mucosa, submucosa, muscularis, and adventitia (Figure 1). The tunica mucosa of the cervical and thoracic parts of the esophagus has very thick layers showing numerous huge mucosal folds covered by very thick keratinized or non-keratinized stratified squamous epithelium which contain numerous excretory common ducts of the esophageal gland. In the cervical part, this epithelium reveals a very thick superficial cell layer of parakeratosis (Figures 2 and 3). The abdominal part of the esophagus displayed a very thick wall and had a narrow lumen. Its mucosa showed a few low epithelial folds which were covered by non-keratinized stratified squamous epithelium (Figure 4). The epithelial height or thickness of the cervical, thoracic, and abdominal parts were 221.95 ± 3.41 µm, 212.46 ± 5.38 µm, and 173.15 ± 4.09 µm respectively.

The current study reveals that the lamina propria of the cervical part of the esophagus is merged with the tunica submucosa due to the absence of muscularis mucosa which is composed of dense irregular collagenous connective

showing little scattered groups of esophageal glands (Figures 2 and 5). At the same time, the thoracic part of the smooth muscles of muscular mucosa appeared as scattered bundles (Figure 3).



Figure 1: Cross section of esophagus- cervical region (Dog) shows (Black arrow) mucosal fold, (Asterisk) epithelium, (Red arrow) fibrous tissue, (S) submucosa, (Blue double arrow) thick inner longitudinal layer, (Red double arrow) middle circular layer of striated muscle fibers of tunica muscularis. H&E stain 4x.

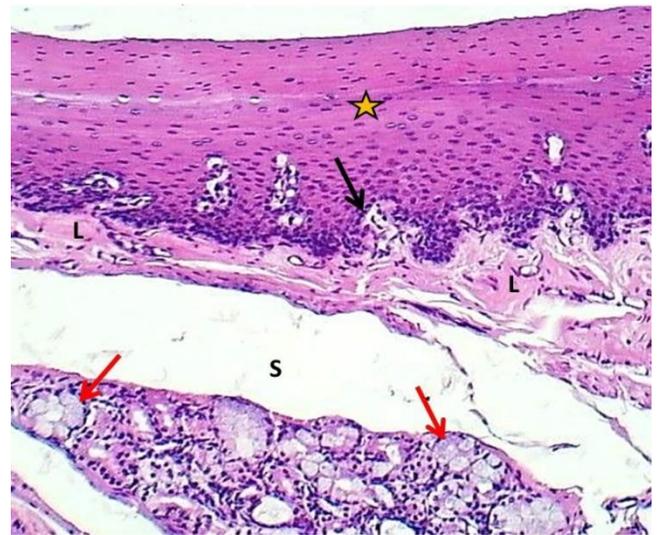


Figure 2: Section of mucosa of esophagus at cervical region (Dog) shows (Asterisk) non-keratinized stratified squamous epithelium, (Black arrow) common duct of esophageal gland, (S) submucosa, (Red arrows) submucosal esophageal glands. H&E stain 10x.



Figure 3: Section of mucosal fold of esophagus-Thoracic region (Dog) shows: (E) thick stratified squamous epithelium, (Black arrows) para keratinized layer, (L) scattered bundles of smooth muscle of muscularis mucosa (Red arrow). H&E stain 10x.

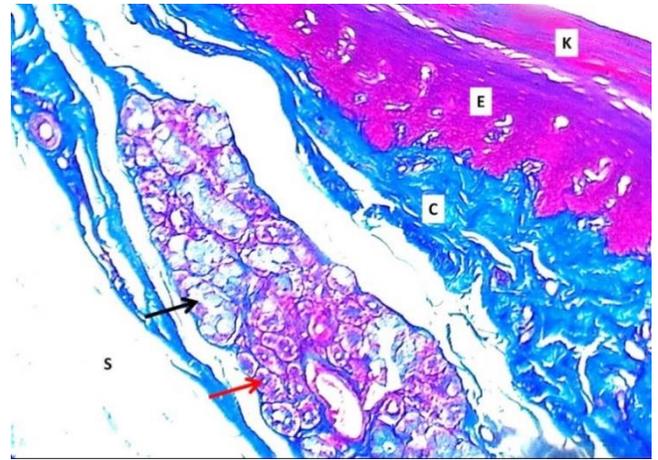


Figure 5: Section of mucosa and submucosa of esophagus at cervical region (Dog) shows (K) keratinize layer of (E) stratified squamous epithelialis, (C) collagen bundles of lamina propria, (Black arrow) mucous alveolus (Red arrow) serous acini of esophageal gland, and (S) submucosa. Masson's trichrome stain 10x.

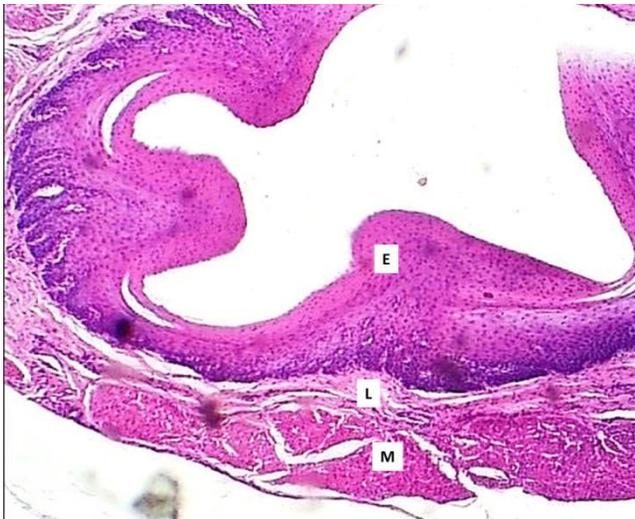


Figure 4: Section of esophagus -abdominal region (Dog) shows: (E) epithelial folds, (L), non-glandular fibrous lamina propria, (M) tunica muscularis. H&E stain 4x.

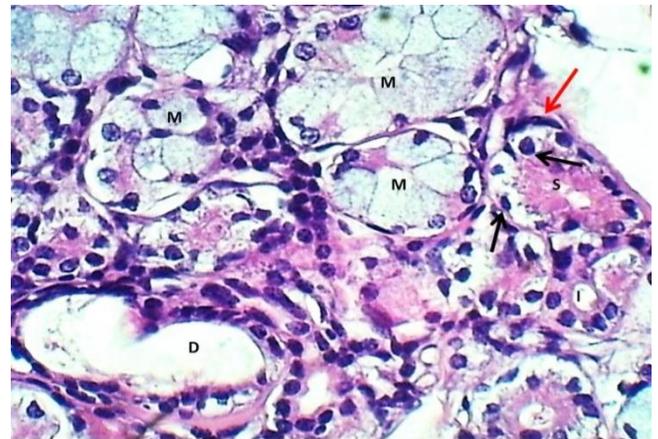


Figure 6: Section of compound tubular mucoserous gland-esophagus (Dog) shows (M) mucous alveoli, (D) duct, (S) mixed serous acinus, (Black arrows) mucous demulcans, (Red arrow) myoepithelial cells. H&E stain 40x.

In local breed dogs, the esophageal glands are compound tubular mucoserous type constructed of numerous predominated large mucous alveoli and little serous acini which are surrounded by a demilune-like mucus (Figure 6). With the combination of Alcian blue (pH2.5)-PAS stain, the esophageal glands reveal dark bluish color denoting strong acidic mucopolysaccharide within the mucous alveoli and light blue color within serous acini that show weak acidic zymogen granules (Figure 7).

The tunica muscularis of the esophagus at the cervical and thoracic parts composes of three layers: thick inner longitudinal, thick middle circular, and very thin outer longitudinal layers of striated muscle fibers (Figure 8). On the other hand, the muscularis of the esophagus in the abdominal part is composed of two layers: very thick inner circular smooth muscle fibers and very thick outer layer bundles of smooth muscle fibers (Figure 9). The thickness of the tunica muscularis of cervical, thoracic and abdominal parts are $568.76 \pm 6.90 \mu\text{m}$, $703.29 \pm 7.54 \mu\text{m}$, and $338.98 \pm 7.26 \mu\text{m}$ respectively.

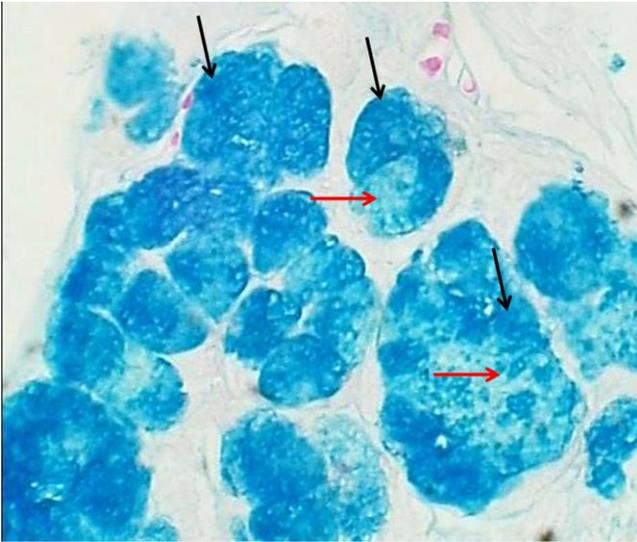


Figure 7: Section of esophageal glands (Dog) shows (Black arrows) mucous acini secreted acidic mucopolysaccharide and (Red arrows) serous acini secreted acidic zymogen granules. Combine Alcian blue (pH 2.5)-PAS stain 40x.

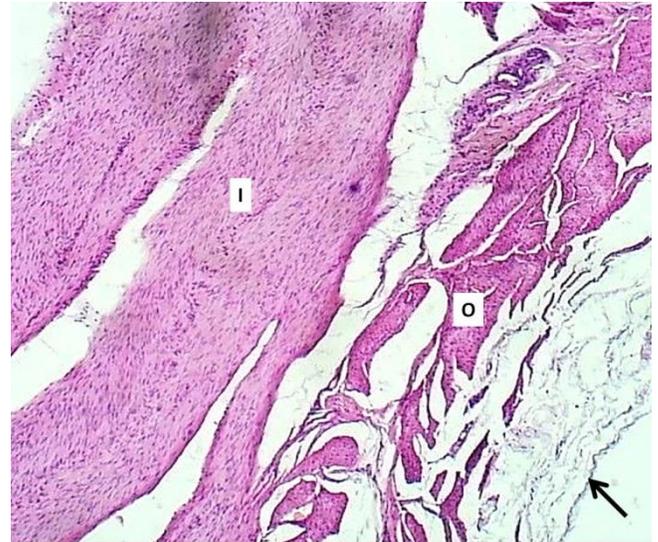


Figure 9: Cross section of esophagus- abdominal region (Dog) shows (Arrow) loose connective tissue covered by mesothelium of tunica serosa, (I) thick inner circular layer and (O) thin outer longitudinal layer of smooth muscle fibers of tunica muscularis. H&E stain 4x.

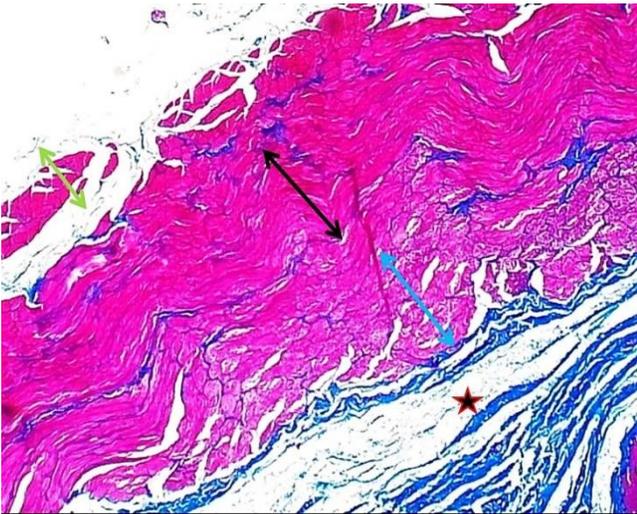


Figure 8: Cross section of esophagus- cervical region (Dog) shows (Blue double arrow) thick inner longitudinal layer, (Black double arrow) middle circular layer and (Green double arrow) thin outer longitudinal layer of striated muscle fibers of tunica muscularis. H&E stain 4x.

Discussion

In local breed dogs, the esophageal mucosa is thick and folded at the cervical and thoracic parts and is covered with very a thick keratinized to non-keratinized stratified squamous epithelium, while the abdominal part of the esophagus has a thick wall and covered by non-keratinized stratified squamous epithelium. The non-keratinized esophageal epithelium is recorded by Berghes *et al.* (14) in Guinea pigs, and by Devi (15) in humans and dogs. The keratinized type is seen in small ruminants (15,16) and Guinea pigs (17). The current results reveal that the epithelium is very thick in the upper two-thirds of the esophagus which is similar to that reported by Kadhim (18) who mentions that the cervical part of the esophagus of Grey Mongoose has the thickest epithelium composed of 8-12 cell layers, while in the thoracic part of the esophagus is 6-8 cell layers, and at the abdominal part, the epithelium is composed of 4-6 cell layers. Also, in Agouti (19) and the study results in goats, the epithelium is thicker in the thoracic part $417.5 \pm 42.11 \mu\text{m}$ as compared to a cervical portion $337.2 \pm 34.4 \mu\text{m}$, and the abdominal closed rumen $308.7 \pm 27.2 \mu\text{m}$. the variance in thickness can be related to the natural carnivore's hard diet. In humans, the thickest epithelium of the esophagus is in the upper part and characterized by numerous peg-like protrusions of the lamina propria indenting the epithelium (15). The current study suggests that the peg-like protrusions of the lamina propria are connected to the gland duct system. The esophagus of the local breed

dogs displays numerous longitudinally oriented mucosal folds which are similar to that mentioned by Davi (15). Similarly, the esophagus in one-humped camels shows smooth mucosa with no folds (7). The mucosal folds in carnivores are related to the protection from damage by hard food. On the other hand, this feature allows expansion as the food goes down to the stomach (20,21).

The current study of the lamina propria of the cervical part of the esophagus reveals the absence of the muscularis mucosa, while in the thoracic part, the muscular mucosa appears as scattered bundles. Similarly, the muscularis mucosa of Grey Mongoose esophagus which is thinly scattered interrupted smooth muscle fibers becomes thick in the middle part of the esophagus (18). It forms an interrupted single layer of smooth muscles in goats (16) and buffalo (22), while the muscular mucosa is prominent along the entire length of the esophagus of agouti (19). However, in dogs, the muscularis mucosa is a smooth muscle fiber layer in the caudal part of the esophagus only. Ali *et al.* (23) refers to the presence of the muscularis mucosae in the caudal part of camels. Meanwhile, Hussein *et al.* (7) records that in one-humped camels, the muscularis mucosa is extended along the entire length of the esophagus.

In local breed dogs, the esophageal glands are compound tubular mucoserous types constructed of numerous predominated mucous alveoli and little serous acini and surrounded by demilune-like mucus. These glands are mentioned by Hussein, *et al* (7) in one-humped camels. The esophagus which is of purely mucous type is characterized by marked and massive esophageal glands distributed along the entire length of one-humped camels. Such mucous glandular tissue is also observed in dogs (24,25). Similarly, it is recorded in Grey Mongoose (18), in Guinea pigs (14), in goats (16), in sheep and oxen (22). The esophageal glands are not observed at any level of the esophagus. The esophageal glands in the esophagus are related to the regurgitation reflex present in canines. The mucus secretion may have a protection aid for the mucosa against the acid pH in carnivores' stomachs. The mucous secretion forms a protective barrier for the esophageal mucosa (10). The lamina propria of the entire length of the esophagus lacks glandular tissue. The results of the present study reveal that the esophageal glands combined with Alcian blue (pH2.5)-PAS stain show dark bluish color which denotes strong acidic mucopolysaccharide within the mucous alveoli and light blue color within serous acini that shows weak acidic zymogen granules. The differences in the secretion of the esophageal gland are associated with the type and food habits of camels unlike those in carnivores. In the esophageal stomach junction, there are simple tubular serous and mucous esophageal glands. These glands are located in lamina propria and few of them extend into the submucosa. They are located at the site of epithelial changing from squamous to the columnar epithelia (18). In local breed dogs,

the muscularis externa of the esophageal cervical and thoracic parts is composed of striated muscle fibers, while the muscularis externa at the abdominal part is composed of two layers of smooth muscle fibers. These results are relatively similar to the tunica muscularis of cervical and thoracic parts of the esophagus of Grey Mongoose which is composed of inner circular and outer longitudinal layers of striated muscle fibers measured as 170 μm and 200 μm in thickness, and turn into smooth muscle fibers in the caudal part of 315 μm thickness (18). These results are also similar to the tunica muscularis of the esophagus humans (15). However, the current results disagree with those seen in esophagus of one-humped camel (7) dogs (15). Naghani and Andi (26) who state that the tunica muscularis of the esophagus is entirely composed of a striated type of muscle. On the other hand, the tunica muscularis of agouti is made up of inner circular and outer longitudinal smooth muscle fibers (19). In horses and pigs, the muscularis consists of two layers that appear as an obliquely striated muscle in the cervical part of the esophagus, turn into a spiral pattern in the thoracic part and are arranged into an inner circular and an outer longitudinal layer of smooth muscle in the abdominal part. In ruminants, the muscularis is a striated type throughout the length of the esophagus (21). In Guinea pigs, the muscle layer is a striated type that turns into a smooth type close to the stomach in order to form the gastric sphincter (14). In goats, the tunica muscularis takes the form of a thick inner circular and a thinner outer longitudinal layer of striated skeletal muscles (16).

Conclusion

The studied esophagus shows considerable histological similarities with that in the other mammals which makes this species reliable as an experimental model for digestive tests and investigations.

Acknowledgment

The authors would like to acknowledge Mr. Muoaid Fadil and the staff assistants in the laboratory of histological techniques at the Department of Anatomy, Histology and Embryology- College of Veterinary Medicine/the University of Baghdad.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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دراسة نسجية ونسجية كيميائية للمريء في الكلاب المحلية

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الخلاصة

تستخدم الدراسة ثمان عينات من مري الكلاب المحلية وذلك لمعرفة الصفات الكيميائية النسجية للمري. تم تحضير العينات بتقنية الطمر بشمع البرافين وصبغت المقاطع النسجية بصبغة الهيماتوكسيلين والايوسين وصبغة الماسون وصبغة الاليشيان (أس الهيدروجيني ٢,٥) المزوجة مع صبغة شيف الايودي. تبين النتائج أن الغلالة المخاطية للمريء في الكلاب تتكون من طبقات مخاطية مغطات بظهارة حرشفية المطبقة متقرنة وغير متقرنة وكانت تحتوي على عدد من قنوات الإفراز الرئيسة للغدد المريء. يبلغ سمك الظهارة للجزء العنقي والصدرى والبطني للمريء $3,41 \pm 2,11,95$ مايكرومتر و $5,38 \pm 2,12,46$ مايكرومتر و $4,09 \pm 1,73,15$ مايكرومتر على التوالي. تبين النتائج أن الغلالة المخاطية للجزء العنقي للمريء خالية من الطبقة العضلية المخاطية بينما يظهر الجزء الصدرى للمريء عضلات لمساء على شكل حزم منتشرة. وتظهر النتائج أن عدد المريء في الكلاب هي من النوع المركب الأنبوبي المختلط ذات الإفراز المخاطي والمصلي وباستخدام صبغة الاليشيان مع صبغة الباس تظهر الغدد تفاعل حامضي قوي للإفرازات المخاطية متعدد السكريات في الحويصلات المخاطية بينما تظهر الأسناخ المصلية للغدد تفاعل حامضي ضعيف. وتتكون الغلالة العضلية من عضلات هيكلية مخططة في الجزء العنقي والصدرى للمريء بينما في الجزء البطني للمريء تكون العضلات لمساء. ويبلغ سمك الغلالة العضلية للجزء العنقي والصدرى والبطني $7,54 \pm 7,03,29$ مايكرومتر و $6,90 \pm 5,68,76$ مايكرومتر و $7,26 \pm 3,38,98$ مايكرومتر على التوالي.