Comparative Histological Study On The Tracheas Of Goose, Duck And Pigeon

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ABSTRACT

Twenty-five healthy male and female birds (8 of goose, 8 of ducks an 9 of pigeons) were used in this work. Tracheas of them were fixed and processed till 7 micrometer thick paraffin sections were prepared and stained with different stains. The results revealed that tracheas of goose and duck contained ossified circular overlapping rings, while those of pigeons contained non-ossified cartilaginous rings. The diameters, length and thickness varied in different studied species.

The tracheal skeletal muscles attached to the lateral sides of tracheal rings.

The lining epithelium of all tracheas was respiratory type with fine goblet cells that were alcianophilic or PAS positive. In some areas were present intraepithelial acini in goose and duck. In pigeon, the intraepithelial acini increased in size and bulged deeply in the propria but there were no submucosal glands in all studied birds.

The ossified regions of the tracheal rings of duck and goose revealed bone marrow spaces with hematopoietic tissue or even adepocytes.

Tracheal rings were connected with each other by fibrous annular ligaments with silver impregnation the reticular fibers were progressively present in the tracheal rings, muscles and adventitia.

The lacunae of chondrocytes of non-ossified regions of tracheal rings in duck and goose showed alcianophilia. Other results were mentioned and discussed.

INTRODUCTION

The tracheas of different bird species attracted many authors to study their anatomical and microscopical pictures, to investigate their significant difference in different species.

Trachea of birds extends from the larynx (which is devoid of vocal cords) to above the base of the heart where it bifurcates into 2 main bronchi (1). It is related to 4 muscles in sternohyoidus, domestic fowls, viz: cleidotrachialis, sternotrachialis and trachialis lateralis (2). The number of tracheal rings of 150-165 as circular ibis reached to cartilaginous ones, that began dorsal to oesophagus then shefted to left and continued till the thoracic inlet (3). The lining epith. of trachea was of respiratory type with mucous glands (1, 4). The latter authors stated that the glands of mucosa are intraepithelial clusters while it has been described those glands as mucous crypts (9).

None of the previous authors pointed to the histochemical reaction of these glands, so this work as a trial to reveal this reactive nature, the number of tracheal rings their dgrees of ossification, width and length of tracheas were also our aim of investigation in the studied species.

MATERIAL AND METHODS

A twenty five apparently healthy domestic birds of both sexes were used in this study, 8 goose one year old, 8 ducks aged one year and 9 pigeon 4 months old. Anatomically 2 of each were used and remainders after slaughter, their tracheas were eviscerated immediately, fixed in buffered neutral formalin 10% or Bouin's fluid, processed till 5-7 micrometer thick paraffin sections ($\boldsymbol{6}, 7$) were prepared and stained with different stains including:

Hematoxylin and Eosin, PAS, alcian blue method, weigert's elastic st. or in combination with Van Gieson's st., Crossmon's trichrome st. and silver impregmenation. The tracheas of each species were divided into pieces (cranial, middle and caudal) before fixation.

RESULTS

Tracheas of both goose and duck were grossly similar as seen on dorsal view to be forming articulating rings, the trachea opens cranially on larynx as a slit-like opening in the floor of the pharynx, and caudally end by syrinx(caudal larynx). The last 5 tracheal rings in both goose and duck share in the formation of syrinx. The bifurcation of the trachea occurs above the base of the heart into two main primary bronchi each for lung on the right and left sides.

The lengths of tracheas of the studied species were 30 cm in goose, 15-17 cm in ducks and 7.9 cm in pigeons. The diameters were 1.1 x 0.55 cm in goose, 0.9x0.7 cm in duck and 0.3 cm in pigeon, and the thickness of tracheal wall was 2 mm in goose, 1.5 mm in duck and 0.4 mm in pigeon.

The tracheal rings in goose and duck were overlapping with each other where half of the rings were successively appeared at one side while the other halves were successively hidden at the other sides as seen in the representating diagrammatic drawing(fig.1). The circular tracheal rings of pigeon were not overlapping but present in a successive manner. The rings were connected by annular ligaments in all studied species.

The tracheal rings of goose and duck revealed constricted (dorsal&ventral) regions between the apparent and hidden halves of each tracheal ring (fig.1), those constricted regions are absent in those of pigeon.

Although the tracheal muscles in birds appeared as 4 pairs related to the lateral aspects of trachea and attached at different levels along the whole length of trachea, those muscles cannot be represented collectively at one level of cross or longitudinal sections.the 4 pairs of tracheal muscles are : sternotrachealis, trachealis lateralis, trachiohyoideus and cleidotrachealis.

The lining epithelium of trachea of all studied species was of pseudostratified columner ciliated type (respiratory type) with many alcianophilic goblet cells or in the form of intraepithelial mucous secreting glands (fig.2), hence there are 3 types of epithelial cells could be discerned , viz, columnar ciliated, basal and mucous secreting cell types.

No muscularis mucosa could be detected in all studied species, so propria and submucosa formed a continous layer that was densely packed sub-epithelially but loosely interwoven depply near the tracheal rings. This layer revealed many lymphocytes but no lymph nodules, rich in elastic and reticular but fewer collagenic fibers (fig.3).

In goose

Every tracheal rings of goose was highly ossified leaving only small cranial and caudal edges (fig.4) or plates.

The annular ligaments connected the cranial and caudal edges were rich in reticular fibers (fig.5).

The cartilaginous plates or edges on both cranial and caudal aspects of each tracheal rings showed PAS positive matrix and alcianophilic lacunae of chondrocytes in these places (fig.6).

Both cartilaginous and ossified portions of each ring were invested by continous dense perichondrium and periosteum respectively (fig.7).

In cross section of the tracheal rings appeared thinner than the case of longitudinal section, where in the latter stage the ring appeared thicker especially in the central region of each ring.

The overlapping of tracheal rings in a cross section is apparent i (fig.8), where the epithelium is followed with thin condensed propria-submucosa without glands and two overlapping tracheal rings, followed with the loose adventitia.

Through the cross section of tracheal rings the more massive part was the central ossified part of each ring (fig.9), this ossified part revealed wide irregular bone marrow spaces lined with delicate endosteum, also smaller bone marrow spaces were discerned (fig.9). The bone marrow spaces were rich in adipose tissue (fig.10).

The outer surface of tracheal rings of loose connective tissue through which are attached the skeletal muscle: trachealis lateralis(fig.11) or any of the other muscles according to the level of section.

The bony portions of tracheal rings showed very developed bony lamellae with well-established osteocytes (fig.2, 9, 11).

In ducks

The lining epithelium of trachea is the same as in goose of respiratory type including intraepithelial glands or scattered goblet cells (fig.12).

The propria-submucosa contained no glands (fig.13), but contained numerous lymphocytes and argyophilic reticular fibers, and the osseus regions of tracheal rings revealed argyophilia (fig.14).

In combination of PAS-alcian blue stain, it revealed alcianophilic intraepithelial mucous glands while the propria-submucosa was PASpositive (fig.15).

The skeletal muscle bundles attached to the tracheal rings were interlaced with collagenic ones, hence the colour of Van Gieson's st. gave both red and yellow colours (fig.16).

The hidden two edges of two successive tracheal rings are attached by annular ligaments to the internal surface of the outer covering ring that hides the previously mentioned two edges (figs 17&18).

As well, the connective tissue of the perichondrium of the hidden edges is continuing with the propri-submucosa that present internal to the covering osseous portion of the outer tracheal ring (fig.19).

The osseous portion of the tracheal rings contained less adipose tissue in the irregular bone marrow cavities (fig.20) in comparison to those of goose.

The bony lamellae of the osseous portions of tracheal rings were more developed in ducks than those of goose (fig.21).

The osteocytes of the osseous portions were elongated parallel to the circumference of the ring, so in longitudinal sections those cells appeared as spheroid bodies (fig.20, 21).

In pigeons

The lamina epithelialis is thicker than in both goose and duck due to thicker intraepithelial glands of mucous type, which are extending deeply in the subepithelial regions of propria-submucosa, the epithelium of the respiratory type as both goose & ducks (fig.22)

The goblet cells and intraepithelial glands were slightly alcianophilic (fig.23). The mucous secreting glands were bulged deeply in the propria-submucosa with vaculated cytoplasm (fig.24).

The complete circular tracheal rings were completely cartilaginous, the cartilage revealed argyophilic strands in the matrix as also rich reticular fibers in propria-submucosa as well as adjacent to the epithelium were present dense reticular fibers (fig.25)

The tracheal cartilages revealed interdigitation or overlapping with each other, and the cartilages were invested in thick perichondrium (fig.26).

There is a thin interrupted layer of connective tissue in the propria-submucosa instead of the muscularis mucosa (figs.22-25).

There were no significant histological differences between males and females in all studied species.



Fig.1: Diagrammatic representation of overlapping tracheal rings of goose and duck(surface dorsa view).



Fig.2: Photomicrograph of cross section in traches of goose, showing lining respiratory epithelium with intraepithelial glandular units(ep), dense propria and loose submucosa (Pm), bony(b) and tracheal skeletal muscle(k). H&E st.,X100





Fig.3: Photomicrograph of cross section in traches of goose showing intraepithelial alcianophilic glands and goblet cells(arrows), dense propria, loose submucosa(pm), compact bone(b) as ossiefied part of tracheal rings, tracheal muscle (m). alcian blue-PAS combination

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Fig.4: Photomicrograph of longitudinal section in trachea of goose, showing free cartilaginous edges (arrows), ossified part(b), perichondrium and periosteum (p), longitudinal skeletal muscle bundles(m). H& E st.,X100

Zag. Vet. J.



Fig.5: Photomicrograph of cross section in trachea of goose, showing reticular fiber distribution, more concentrated in the annular ligament between tracheal rings as an argyophilic fibers (1), in epithelium(ep), fewer in propria-submucosa(pm). Silver impregnation method, X100.



Fig.6: Photomicrograph of longitudinal section in the trachea of goose showing alcianophilic cartilaginous lacunae(c) and PAS positive matrix(m), perichondrium(p).alcian blue- PAS combination, X400.



Fig.7: Photomicrograph of longitudinal section in the trachea of goose showing bony(b) and cartilaginous(c) portions of tracheal rings, tracheal muscle(m) bone marrow(bm). H& E st.,X100

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Fig.8: Photomicrograph of cross section in trachea of goose showing overlapping 2 adjacent cartilaginous rings© and bony part(b) of another ring, epithelium(ep), skeletal muscle(m). H&E st, X40.

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Fig.9: Photomicrograph of longitudinal section in trachea of goose showing the more massive part of tracheal ring in bony(b) with irregular bone marrow spaces(bm) rich in bone marrow constituents. H&E st,.X150.





Fig.10: Photomicrograph of longitudinal section in trachea of goose showing bone marrow rich in adipose tissue(ad) and bone marrow cellular constituents(c). H&E st,.X400.

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Fig.11: Photomicrograph of longitudinal section in trachea of goose showing bony part(b), longitudinal skeletal muscle bundles(m), periosteum(pr) and adventitia(ad). H&E st,.X100



Fig.12: Photomicrograph of longitudinal section in the trachea of duck showing the lining epith. Of pseudostratified columnar ciliated epith with goblet cells(g), groups of mucussecreting intraepithelial units(gc), dense propria, loose c.t submucosa of. without muscularis mucosae(pM). H&E st.,X400

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Fig.13: Photomicrograph of longitudinal section in the trachea of duck showing propriasubmucosa(pm) with no glands and deeply loose c.t. . osseous parts (os) of tracheal rings, lining respiratory epith.(ep). H&E st.,X400.



Fig.14: Photomicrograph of longitudinal section in the trachea of duck showing argyophilic picture of tracheal ring in the lining epith.(ep), slighter in propria-submucosa(pm), stronger in compact bone of the ossified parts of tracheal ring(b), longitudinal skeletal muscle bundles(m). silver impregnation, X100.





Fig.15: Photomicrograph of longitudinal section in the trachea of duck showing alcianophilia of the intraepithelial glandular units and goblet cells of the lining epith. Of trachea(arrows), also propria-submucosa is alcianophilic(pm). Alcian blue-PAS combination, X400.



Fig.16: Photomicrograph of longitudinal section in the trachea of duck showing skeletal muscle bundles(m) in adventitia of trachea, lining epith. In yellow(arrows), bony part of tracheal ring in red, irregular bone marrow cavities(s). Van Gieson's st., X40.

Zag. Vet. J.



Fig.17: Photomicrograph of longitudinal section in the trachea of duck showing the overlapping of 3 tracheal rings; 1,2&3 where the 2 edges of 1&3 are hidden by the massive bony part of 2, caudal cartilage edge of 1(cu) and cranial cartilage edge of 3 (cr), lining epith.(ep). Propria-submucosa(pm), annular lig. Attaching the tracheal edges(an). H&E st., X100.



Fig.18: Photomicrograph of longitudinal section in the trachea of duck showing overlapping of 2 successive tracheal rings 1(outer) and 2 (inner) cartilaginous edges of them respectively(a&b). lining epith. Of trachea (ep), adventitia (ad) without apparent muscle bundles, annular lig(an). H&E st.,X100



Fig.19: Photomicrograph of longitudinal section in the trachea of duck revealing overlapping tracheal rings perichondrium (pr) and periosteum (po), propria-submucosa(pm), lining epith. Of trachea(ep), annular lig(an). H&E st.,X100



Fig.20: Photomicrograph of longitudinal section in the trachea of duck revealing bony lamellae(1) of ossified region of tracheal ring with rare adipose tissue in the bone marrow spaces comparatively with those of goose(c), periosteum(po). H&E st., X400





Fig.21: Photomicrograph of longitudinal section in the trachea of duck revealing lamellae(1), haversian canal (c), arrangement of osteocytes (os) around the central canals, more distinct than in goose. H&E st.,X400



Fig.22: Photomicrograph of cross section in trachea of pigeon revealing lining resp. epith.(ep) with intraepithelial mucus-secreting goblet cells and glandular units bulging to propria(pr), overlapping hyaline cartilaginous rings(1.2), adventitia(ad). H&E st., X100

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Fig.23: Photomicrograph of cross section in trachea of pigeon revealing faint alcianpohilia of goblet cells(g) some alveolar glands(g), but other alveoli were alcianophilic(pb), tracheal rings(r) with faint alcianophilia in chondrocytesthin interrupted c.t. layer(1). Alcian blue st., X100



Fig.24: Higher magnification of tracheal epith.of pigeon revealing goblet cells(g), bulging mucous alveoli towards the propria (gl), propria-submucosa(pm). H&E st., X400

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Fig.25: Photomicrograph of cross section in trachea of pigeon revealing argyophilia of epith.(ep), no in alveolar units(gl), condensed ret, fibers between the glandular units and perichondrium of tracheal cart. rings, between the chondrocytes, adventitia(ad), thin interrupted C.T.layer(1). Silver impregnation, X100.



Fig.26:Photomicrograph of cross section in trachea of pigeon revealing completely hyaline cartilage tracheal rings(r) with thick perichondrium(pr), loose connective tissue propriasubmucosa(pm) that invest the glandular alveolar units only in its superfacial regions, adventitia(ad), thin interrupted C.T. layer(1). H&E st.,x1

DISCUSSION

Our results relating to the bifurcation of trachea on the base of heart in the thoracic cavity in the three studied species simulate that recorded in fowls (3, 5) but differ from that cited in penguins (9), where the tracheal bifurcation occurs in the cranial end of the neck without forming an obvious syrinx.

The resonating structure (syrinx) is present in the three studied species to form bulla lympanifermis and its presence as fused 6 rings of trachea of drake (male goose) (I), but it is denied to that found in ibis (3).

Regarding the number of tracheal rings and their ossification degrees, they varied in number according to species studied (180-185 in goose), (137-140 in duck) and (55-60 in pigeon). In ibis found the number of tracheal rings was 150-165 rings (3), while in fowls (8) were 100-130, (2) or 108-126 which may be ossified (2).

The lengths of tracheas in the studied species were: 30 cm in goose, 15-17 in duck and 7-9 cm in pigeon, while previous study (3) in ibis did not mention the thickness of the wall. In all studied species those were: $10.1 \times 5.5 \text{ mm}$ and 2 mm in goose, $0.9 \times 0.7 \text{ mm}$ and 1.5 mm in duck and 2 mm and 0.4 mm in pigeon, those dimentions were not recorded in the available literature.

The overlapping of partial parts of tracheal rings in studied goose and duck tracheas is similar to that recorded in domestic birds (1), fowls (2, 3, 8) and ibis (3).

Regarding the respiratory epithelium that lines the trachea of studied bird species it is the same as those mentioned through the available literature, in chicken tracheal epithelium where it is formed of three cell types (11). Moreover, the rate of ciliated and non-ciliated cells as well as the degree of secretion could be variable according to air pollution in some birds and some mammals (12).

The propria under the respiratory epithelium of trachea in the studied species which was of dense connective tissue without glandular units, this was consistent with those in fowls (8), ibis (3) but inconsistent with those in Fayomi fowls (10).

The ossification of cartilaginous rings that could be detected in this work including tracheas of both goose and duck are of various degrees, but were absent in trache of pigeon, hence this ossification is in agreement with finding in ibis (3) and domestic fowls (13-15), while the non-ossification of pigeon traches is in a line with that in quail (16). On the other hand, the tracheal rings of Fayomi fowls were completely cartilaginous (17).

The muscles studied in this work (fig 11, 16) were sternotrachealis and tracheolateralis as those studied enzymatically in ducks (I8). They were passing longitudinally along the length of trachea laterally at varying degrees of attachment to tracheal rings. In domestic fowls similar structure was cited (2). The muscles having different functions including diglutations and voice production (I9), as well, their relation to the attachment to lateral aspect of trachea (20, 21).

The mucus-secreting glandular units of the tracheal epithelium were more numerous in pigeon, where pigeon possesses large numbers of mucin-producing cells (22). Domestic fowls are taken as source of tracheal mucinproducing cells for development of a primary cell culture model system because this species possesses a large population on intraepithelial mucin producing cells and it lacks submucosal glands. The latter finding was confirmed by this investigation in absence of any glands in the submucosa of the studied species.

The alcianophilic activity of the intraepithelial goblet cells or glands of the respiratory epithelium in studied species as secreting acidic mucin is in a line with the findings in domestic fowls (22).

The bony lamellae of osseous portions of tracheal rings were more organized in duck than in goose, hence, the rings of duck were harder, they needed more time for decalcification during processing of specimens than with those of goose.

REFERENCES

- 1. Nickel, R., Schummer, A. and Seiferle, S. (1977): Anatomy of the domestic birds. 1st Ed., Verlag Paul Pery, Berlin, Hamburg.
- 2. Mclelland, J. (1969): The anatomy of the rings and muscle of trachea of Gallus domesticus. J.Anat., 95: 651-656.
- 3. Omar, A. and Nousser, H. (1988): Morphology of the trachea and syrinx of ibis (Ardeola Ibis ibis) with attention to their muscels. Acc. In the 11th Egypt .Anat.Soc.
- 4. Samuelson, D.A. (2007): Textbook of Veterinary Histology. Saunders Elsevier Inc., Missouri.
- Trautmann,A. and Fiebiger, J. (1957): Fundamentals of the histology of domestic animals. 3rd Ed.,.Printing Comstock Publishing Associates Isthaca
- 6. Sheehan, D.C. and Hrapchak, B.B. (1987): Theory and practice of Histotechnology, 2nd Ed., Battelle Press, Columbus and Ohio.
- Bancroft, JD. and Stevens, A. (1990): theory and practice histological techniques. 3rd Ed. Churchill Livingstone, Edinburgh, London, Sydney, Melbourne & New York.
- 8. Hodges, R.D.(1974): the histology of fowl. Academic Press, London, New York and Francisco.
- 9. King.A.S. and Mclelland, J. (1975): Outlines of avian anatomy. 1st Ed., Bailliere Tindall, London.
- 10. Abdel-Mohdy, F.S. (1990): some morphological studies of apparatus respiratorius of Fayomi fowl. M.V.SC. Thesis, Fac.Vet, Med., Zagazig University.
- 11. Lai,M.C. and Ibrahim, A.L.(1984): scanning and transmission electron microscopy of normal chiken tracheal epithelia. Poult, Sci.,63(7); 1425-1431.

- 12. Gorriz, A., Liacuna, S., Durfort, M. and Nadal, J. (1994): A study of the ciliar tracheal epithelium on passerine birds and small mammals subjected to air pollution; ultrastructural study. Arch. Environ Contam. Toxicol., 27(1): 137-142.
- 13. Garside, J.S. (1968): ossification of the tracheal cartilages in the fowl. Vet. Rec., 82:470-471.
- 14. Hogg, D.A. (1978): Mineralization of the larynx, trachea and syrinx in domestic fowl. J.Anat., 127(2):658-659.
- 15. Hogg, D.A. (1982): Ossification of the laryngeal, tracheal and syringyal cartilages in domestic fowl. J. Anat., 134(1): 57-71.
- 16. Fitzgerald, T.C. (1969): The coturnix quail anatomy and histology. 1st Ed., The Iowa State University Press, Ames, Iowa.
- 17. Badawy, Y.H., Ewais. M.S., Abbas, A. and Abdel- Mohdy. F.S. (1981), A morphological study of the respiratory system of the adult Fayomi fowl. Egypt. J. Histol., 4(1):105-112
- 18. Gopalakrishnakone, P.(1985): Structure and Innervation of the tracheal muscles of the white Pekin duck.J.Anat.,140:205-219.
- 19. White, S.S (1968): Movement of the larynx during growing in the domestic cook. J.Anat., 103: 390-392.
- 20. King.A.S.(1979): Systima respiratorium: In Nomina Anatomica Avium (ed. A.A. Ituamel, A.S. King, A.M. Lucas, J. E. Breasile & H.E. Evans), Academic Press, London and New York.
- 21. Vanden Berge, J.C. (1979): Myologia, In Nomina Anatomica Avium(ed. As Literature, 14).
- 22. Douglas, W.H.J., Gustafson, A.W. and Aghajanian, J.D. (1982): isolation culture and preliminary characterization of mucin producing cells from trachea of the domestic fowl.Anat.rec..202:285-296.

الملخص العربي دراسة نسجية مقارنة للرغامي في الإوز والبط والحمام

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تم استخدام عدد (٢٥) خمسة وعشرون طائرا (٨ من الإوز ، ٨ من البط ، ٩ من الحمام) من كلا الجنسين • حيث تم بعد الذبح فصل الرغامى من كل طائر وتم قياس الطول والقطر وسمك الجدار وعدد الحلقات لكل نوع من الطيور – وأخرى تم تثبيتها فور ذبج الطيور وتم تمريرها حتى الحصول على قطاعات بارفينية وتم صبغها بالصبغات المختلفة • كما تم عمل إزالة الكالسيوم فى بعض العينات للبط والأوز لتسهيل تقطيعها فيما بعد نظرا لتعظم حلقات الرغامى •

وقد أظهرت النتائج اختلافات واضحة في الطول والقطر وسمك الجدار وعدد الحلقات في الرغامي للطيور المختلفة بأرقام تم ذكرها في النتائج حيث كانت الرغامي في الإوز الأطول والأوسع والأسمك جدارا وفي عدد الحلقات بينما كانت أقلها في الحمام وأوسطها في البط.

كانت حلقات الرغامي متداخلة في الأنواع الثلاثة ولكن في الحمام كانت التداخل غير ماهو حاصل في كل من الأوز والبط وقد وجد التعظم في حلقات الرغامي للبط والإوز ولكن لم يوجد في الحمام .

كانت الرغامي متصلة من الجانبين بعضلات هيكلية مخططة.

كانت الرغامي مبطنة بنسيج طلائي تنفسي مع عدد قليل من الخلايا الكاسية المفرزة للمخاط واسنخة داخل النسيج الطلائي والذي كان أكثر بروزا ووضوحا تجاه الطبقة الاساسية وأكبر في الحمام عنه في البط والإوز ٠

الأجزاء المتعظمة أحتوت على فجوات عظمية بها خلايا أمهات تكوين خلايا الدم وخلايا دهنية ، أما الأجزاء الغضروفية فقد أحتوت على فجوات بها خلايا غضروفية تفاعلت مع الألشيان الأزرق • كانت حلقات الرغامي متصله مع بعضها بأربطة ليفية حلقية •

لم تظهر اختلافات ذات دلاله بين أنسجة الذكور والأناث في الأنواع التي تم دراستها • كانت غضاريف حلقات الرغامي من النوع الزجاجي • تم تدوين النتائج الأخرى ومناقشتها •