

Histomorphological Structure of The Ultimobronchial Gland In The Indigenous Geese *Anser anser* (Gray lage Goose).

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Summary

The indigenous geese have symmetrical pair of ultimobronchial gland, pink in color, lenticular or globular in shape. These glands are located in thoracic inlet, posterior to the parathyroid gland, dorsal to the bifurcation of brachiocephalic trunk. In young birds the left gland usually adheres to the caudal pole of parathyroid gland and usually cranio lateral to the origin of common carotid artery, while the right gland is also related to common carotid artery, but it's separated from caudal pole of parathyroid gland. The gland is supplied by collateral branch from common carotid artery and innervated by branch from the thoracic vegal trunk and directed from the recurrent nerve. The gland is not surrounded by capsule but it's covered by mass of adipose tissue. The parenchyma of gland consists of epithelial component supported by loose connective tissue which mostly composed of network of collagen fibers interrupted by few elastic fibers. The epithelial component of the gland consists of calcitonin producing cell or C-cell, vesicles and accessory parathyroid nodules. The C-cells are arranged in strands or cords. The connective tissue stroma of gland contains various number of follicles or vesicular structures, which are variable in shape, size, structure and secretory activity. In adult birds, the large vesicles were transformed to cystic structure, which occupied the large part of gland.

Key words: Ultimobronchial gland, Indigenous geese, *Anser anser*.

التركيب النسيجي والإشكالي للغدة الغلصمية للوز المحلي

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الخلاصة

يمتلك الوز المحلي زوج متناظر من الغدد الغلصمية ، تتميز الغدة بلونها الوردي وشكلها العدسي أو الكروي . تقع عند مدخل الصدر خلف الغدة جنب الدرقية ، ظهرياً للجذع العضدي الدماغي . تكون الغدة في الطيور اليافعة غالباً ملازمة للغدة الجنب الدرقية خلفياً في الجهة اليسرى ، عند الجهة الامامية الوحشية لمنشأ الشريان السباتي العام . ولكنها تكون ملاصقة له ومنفصلة عن الغدة الجنب الدرقية في الجهة اليمنى . التجهيز الدموي للغدة عن طريق الفرع الجانبي للشريان السباتي العام . أما التجهيز العصبي لها بواسطة فرع من الجذع الودي الحائر بالإضافة الى فرع من العصب الراجع . التركيب النسيجي للغدة يتميز بعدم وجود محفظة محاطة لها ، وتكون مطمورة في كتلة من النسيج الدهني . أما التكوين الخلوي يتكون من ظهارة مسندة بنسيج رابط رخو من الألياف الغروية ، مع قليل من الألياف المرنة . الخلايا الظهارية المسماة بـ (الخلايا السينية) تنتظم على شكل أعمدة أو حبال ، وهذه الخلايا لها القابلية على إفراز مادة (الكالسيتونين) . وهناك أعداد متباينة من التراكيب الجريبية على شكل حويصلات ذات الاحجام والأشكال المختلفة منتشرة في متن النسيج الرابط ، وتتحد عدد من هذه الحويصلات لتكوين تركيب كيسي كبير يشغل معظم المتن في الطيور البالغة .

Introduction

Many breeders had the interest in establishing projects of breeding water birds such as geese and ducks by providing both of appropriate environment and conditions . Thus the domestic geese are well adjusted to live on lands as well as live in ponds and swamps of villages and countryside . The geese become an important source of meat, livers, fats, feather and eggs. The ultimobranchial gland presents in all classes of vertebrates including birds , fish , amphibian and reptiles , and its remains as separated organs during both embryonic and adult life (1, 2& 3), but in mammals this gland is normally fused with the parathyroid gland during development period and disappear in the adult (4&5). The ultimobranchial gland is a source of the hypocalcemic peptide hormone or calcitonin . It is produced by the Para-follicular cells of the parathyroid gland in mammals and by C-cell of ultimobranchial gland in birds and fish (6,7 &8). Calcitonin is peptide hormone that regulates the balance of serum calcium and blocks the transfer of calcium from bone to blood (5,9 &10). Little is known about the morphological, features of ultimobranchial gland of indigenous geese . Therefore the purpose of this study presented to determine the relationship in different morphological structure of this gland, according to the ages.

Materials and Methods

The experimental animals To reach the aim of this study, forty birds from indigenous geese were divided as following :- **A. Adult geese:** Twenty adult geese of indigenous strain (ten males شسي ten females) were obtained from commercial market. Observation of the birds over a period of two weeks before use were performed , followed by post – mortum examination, to confirm that all studied birds were free of any infections or lesions . They were considered to be clinically normal . **B. Young geese:** Twenty young of indigenous strain (ten males & ten females) were obtained after hatching from commercial market and rearing in animals house of college of veterinary medicine / university of Baghdad . Although these birds were sacrificed immediately after collection (one – month age). No birds showed any evidence of overt systematic disease either external or subsequently post–mortem examination, they were therefore considered to be clinically normal . Twenty geese were used for the anatomical observation, ten adult geese (five males & five females) and ten young geese (five males and five females). The following anatomical indices were used in this work as body and gland weight, position of gland diameter of gland blood and nerve supply of gland . The body weight and gland weight were recorded for each bird in the two equal groups by using sensitive balance (Sartorius Electric balance – Made in Germany)¹. The body weight was recorded before the birds were sacrificed. Each adult bird was Electric balance – Made in Germany anesthetized by slow intravenous injection in wing vein by (2ml) xylazin with (2ml) ketamin and the young bird was anesthetized by injecting (0.5ml) xylazin with (0.5ml) ketamin in the left thoracic muscle using syringe of (1cm³) . The birds were left for (2-5 min) to complete anesthesia (11). The bird was placed on its back in a restraining through and the wings and legs were secured. The feathers were plucked from the sternum area and the body cavity was opened . The ribs were cut on both sides and the ribs cage lifted to uncover the heart and its major arteries were clearly visible. The ultimobranchial gland was made visible by inserting a curved, blunt probe behind the most posterior parathyroid, and moving the common carotid artery medially.

The gland was removed by carefully severing the connecting tissue through using of an extra fine micro dissecting scissors with spring action pencil grip handle. The gland was weighed and then used in the latter index of anatomical study. **Diameter of the gland:** The diameter of the gland was measured by using the vernia in each bird in the two equal groups. **Blood supply of the gland:** To investigate the blood supply of gland, eight geese were used, four adult geese (two males & two females) and four young geese (Two males & two females). The anesthetized bird have been given a time to complete bleeding through a pinhole opened in the left ventricle until they died. The birds were injected by using (20ml) syringes attached to catheters and inserted in to the heart. Firstly normal saline was used to wash the blood vessels, followed by a mixture consist of 2 parts ammonium hydroxide to 3 parts latex, carmine stain was added. To prevent the flow of latex glacial acetic acid was added and the heart pinhole was closed by the artery forceps. After the injection, the whole bird body was placed in 10 % formalin for 48hr (11). **Histological study:** Twenty geese, ten adult geese (five males & five females) and ten young geese (five males & five females) were used for the histological observations. The birds were anesthetized as mentioned before, then they were sacrificed. The gland was removed from each bird and fixed in Bouins fluid for 72 hrs. The histological process were done according to Luna (12).

Slides are cut in thickness (5-6) micrometer. Mayer's hematoxylin and eosin, Periodic acid Schiff (PAS), Alcian blue stain (AB) at (PH 2.5) , Van -Gisson stain (12) , Masson trichrome stain were used . Stained slides were examined by using light microscope type olympus.

Results and Discussion

The anatomical result of the present work revealed that the indigenous geese has the symmetrical pair of ultimobranchial gland , small in size and pink in color (Fig 1 and 2.) they are globular or lenticular in shape, similar to other domestic fowl which were studied by (13, 14, 15 & 16) While King and McLelland, (15) mentioned that the gland in domestic fowl is located dorsoventrally and flattened, irregular in shape. In the other hand the left gland is larger than the right gland in both adult and young geese (Fig.2) . Through the table (1 and 2) present data explain the dimension and the weight of gland especially in adult and young geese, but the diameter of the gland instead of the weight have been reported to be 2.5mm in fowl (3, 13 and 15) . In contrast (14) referred to the diameter of the gland in the domestic birds which is proximately 1.5mm . The gland is located in the thoracic inlet, posterior to the parathyroid gland dorsal to the bifurcation of brachiocephalic trunk in area when this trunk is terminated to the common carotid artery and subclavian artery (Fig.1, 3 and 4). Also related to the medial aspect of the vagul trunk , jugular vein and the lateral edge of esophagus, so there is similarity in position to other domestic fowl (15, 16, 17 and 18). White Hodges (19) mention that the ultimobranchial gland in some domestic fowl is located in midway between the parathyroid gland and the lateral edge of the esophagus, or the gland lie partially close or surround the carotid body. In young geese the left ultimobranchial gland adheres to caudal pole of parathyroid gland, craniolateral to origin of the common carotid arteries while the right gland is separated from caudal pole of parathyroid gland. (fig.5). These findings were described only in the adult domestic fowl by (15 and 20) . In indigenous geese, the left and right gland was related to medial edge of the jugular vein but separated from it, so there was disagreement with the previous findings of (21) who mentioned

that in chicken the gland lie very close to jugular vein. In adult females geese, under influence of the age the glands are embedded in adipose connective tissue , while in young bird usually have no adipose tissue and the gland are easily detected.(fig.6). These result were in agreement with (1 and 13) . The blood of the ultimobranchial gland of geese is supplied by a collateral branch from the common carotid artery. These result was mentioned by (17, 22 and 23) in the domestic fowl. This branch before reaching the gland is terminated into two branches, the dorsal branch supply the gland, while the ventral branch terminated to esophagus in both left and right side.(Fig.7) . These result were in agreement with that mentioned by (1 and 14) in chicken.

The present work shows that the glands were innervated by branch from the thoracic vagul trunk and this branch is supplying the lateral side of gland. Other nerve which innervated the gland called recurrent nerve that arise from the vagus trunk. On the right side, the recurrent nerve curves around the root of the aorta and than running cranially to supply the medial side of right gland, while in the left side the recurrent nerve loop around the ligmentum arteriosum and also it's running cranially to supply the medial side of the left gland (Fig.8&9). These result were in agreement with that mention by (1, 24 and 25) disagreement with (17 and 22) which were refereed that the gland receiving nerve fibers only from nearby nodes ganglion. The glands of geese are not surrounded by capsule, but it's covered by a mass of adipose connective tissue.(Fig.10). This mass increase in it's thickness in adult bird and this result was in paralled with other domestic fowl (15, 16 and 19). The parenchyma of the gland generally have diffuse cellular structures and supported by connective tissue stroma .

Connective tissue stroma: The gland has loose connective tissue and mostly composed of network of collagen fibers interrupted by few elastic fibers with out smooth muscle cells also present within the stroma bundles of connective tissue that support the strands or cords of the C-cell and vesicles . These cords infiltrated by adipose tissue and supplied by blood vessels and nerves(Fig.11). Similar description was mentioned earlier in the fowl by (16, 19 and 21). In the other hand the bundles of the connective tissue become thicker in adult birds than the young and these result was in agreement (21) in chicken. **Epithelial component:** The epithelium components of the geese gland consist from different cellular matter, vesicles, parathyroid nodules and cysts. These findings were in agreement with the findings of , (16and19). The main cells present in these study are the eosinophelic calcitonin cells or C-cells. These cells are arranged in strands or cords or also many single C-cells are also present scattered throughout the stroma of gland. However, the C-cells are variable in shape, it could be rounded, oval or polygonal in shape and always closely associated with fine blood capillaries.(Fig.21). These result were in agreement with that mention in the avian (13,15,16,21,26,27,28and29).The present study, in the other hand, shows that the strand of the C-cells in young bird having more calcitonin cells aggregation than in adult birds.(Fig.13&14) Though the calcitonin well be of greater importance needed in young birds. These findings were supported in the fowl by (30and31). Present histological observation has revealed that the C-cells cytoplasm contain variable numbers of large and fine granular bodies. However, some of the C-cells appears pale (light) cytoplasm while the other groups have dark or opaque cytoplasm. The cytoplasm of the pale cells have few and small scattered granules among the transparent cytoplasm. The nucleus of these cells are large and occupy more of it's cytoplasm. However, the cytoplasm of dark cells is occupied by many numbers and variable size of granules. Mostly aggregated at the apical surface of the cell and the

nucleus is pushed at the distal position of cell. (Fig.12). These findings were supported by Velicky (32) and Treilhou-Lahille (16) in chicken. Wheeler (33) suggested that cytoplasm of the C-cell passing through different stages of maturity, due to the C-cells contain numerous secretory granules of hormone calcitonin that is in agreement with other workers. The ultimobranchial gland of both adult and young geese have the same accessory parathyroid nodules. (Fig.15) which is situated at the peripheral. These results were in agreement with (15,19,21 and 34). Each parathyroid nodule is enclosed by a narrow connective tissue capsule. Similar description was mentioned earlier in the chicken by Egawa and Kameda, (35). The tendency of this capsule increases with maturity, so there was disagreement with Isler (21) who showed that this capsule is well developed in young chicken but tends to become reduced with maturity. The strands of intermediate cells pass through the capsule of the parathyroid nodules. These strands enter the stroma of ultimobranchial and connect up with the vesicles. These results showed the interconnection that occurs between the accessory parathyroid nodules and ultimobranchial tissue which was mentioned by (15 and 36). Besides the presence of C-cells, and connective tissue all ultimobranchial glands of the geese are characterized by presence of follicles or vesicular structure in various sizes, shapes and luminal contents. This is related to the degree of maturity and type of activity. Similar results have been reported in the domestic fowl by some investigators (15,37,38 and 39). Most of these vesicles have an empty appearance [Fig. 15]. In contrast, some of these vesicles have differing secretory material granular and cellular debris which show positive reaction with PAS stain [Fig.16]. In young geese, most of the gland stroma is occupied by few numbers of small rounded or irregular in outline vesicles invaded by blood capillaries. (Fig.17). Thereafter, these vesicles gradually increase in number and volume and become large single or groups of cysts in the stroma of adult geese [Fig.18]. This finding was in agreement with findings of Isler, (21); Hodges, (3). However, the vesicles are mostly lined with simple cuboidal cells, similar to the vesicular lining when they have been described in ultimobranchial gland of domestic fowl by Isler, (21); Hodges, (3). Thus the result was in disagreement with Chan, (40) who reported in chicken that the epithelium lining the vesicles include stratified squamous and pseudostratified columnar cells. The type of epithelium lining the vesicles does not seem to be related either to age of bird or the volume of vesicles by Kameda (41), so this result was confirmed by the present study. The connective tissue stroma of ultimobranchial gland in adult geese have well developed large vesicles transformed to cystic structures occupy the greater part of the gland. These cysts are variable in size and shape (Fig.19). However, this cyst is lined with stratified squamous epithelium and the size of this epithelium could be seen with thick walls and have no sign of secretory activity. Similar description was mentioned earlier in the avian by Coull and Hodges, (42), Ito (27) and Kameda (41). Therefore these cysts act as store for a staining fluid in their lumen or these appear as empty structures due to contain substance which is insoluble in different processing fluid (Fig.20).

The ultimobranchial gland, in both young and adult geese, have several dense lymphoid foci found mainly at peripheral part of gland, but their lymphoid tissues occur without germinal centers (Fig.21). These results were in agreement with that mentioned by (15,36 and 37). The diffuse aggregation of lymphocytes (fig.,22) are found in highly organized structure of the birds. However, the histological appearance of these lymphocytes at any particular time will reflect the immunological status Wheeler (33). The ultimobranchial gland have very well developed, numerous fine capillaries can be seen in all part of gland, which frequently located in close proximity to the strands of C-cell. Large bundles of myelinated and unmyelinated nerve fiber

present within the connective tissue stroma of the gland. These bundles distributed in the space around the C-cell (Fig.16).

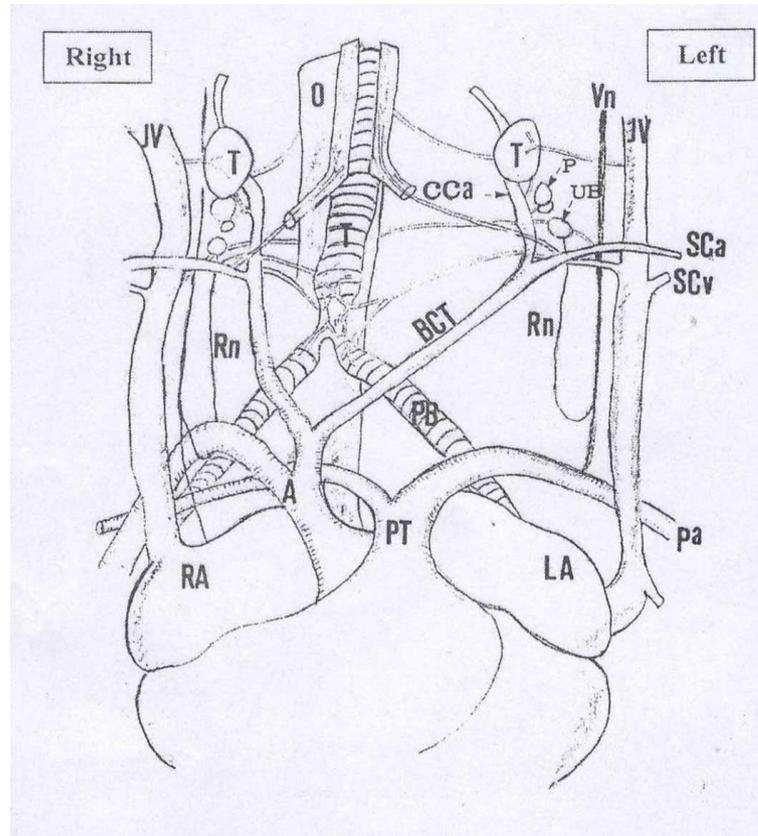
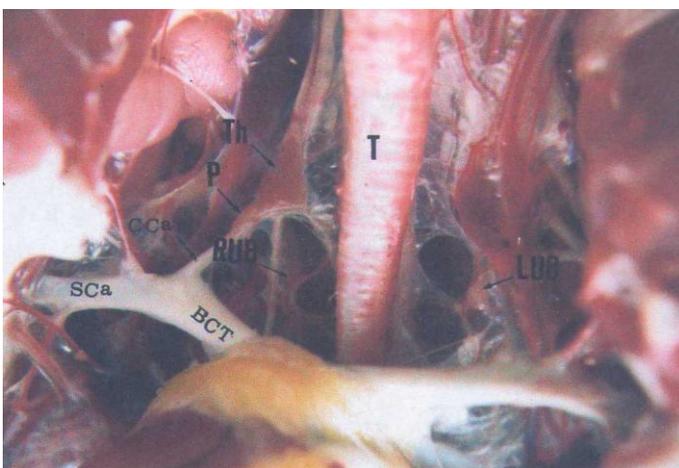
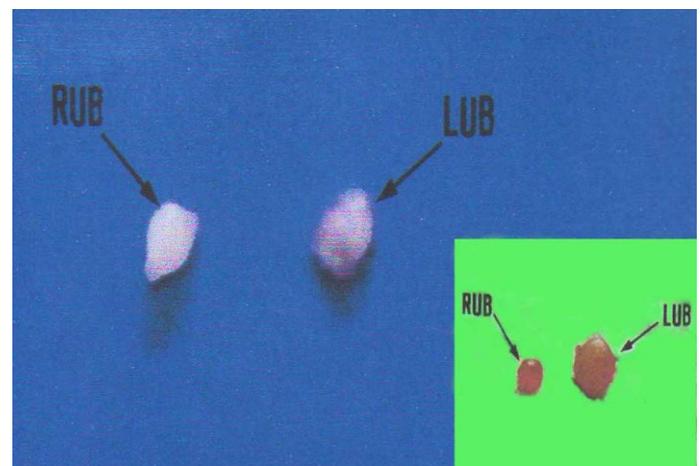


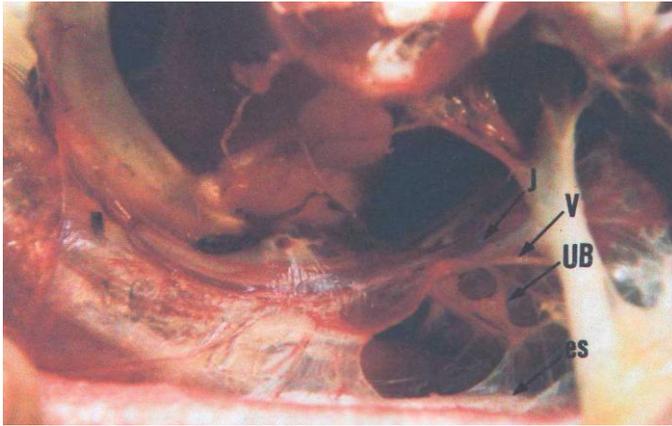
Figure (1): Ventral view of the blood vessels, nerves and position of the ultimobranchial gland at the thoracic inlet of the geese. PT: pulmonary trunk; RA: right atrium; LA: left atrium; A: aorta; JV: jugular vein; BCT: brachiocephalic trunk; CCa: common carotid artery; SCa: Subclavian artery; SCv: Subclavian vein; Pa: Pulmonary artery; Vn: vagus nerve; Rn: recurrent nerve; o:esophagus; T: Trachea; PB: primary bronchus; T: Thyroid gland; P: parathyroid gland; UB: ultimobranchial gland .



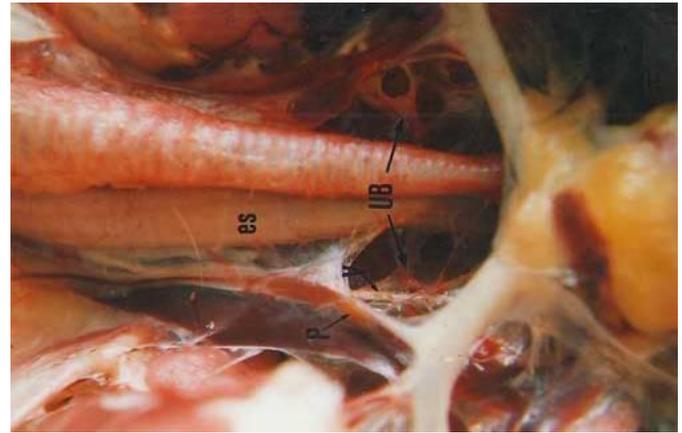
(Fig.3): Photograph shows the position of ultimobranchial gland in adult geese, left ultimobranchial (LUB);right ultimobranchial (RUB); Thyroid gland (TH); Parathyroid gland (P);Trachea (T); Brachiocephalic trunk (BCT); Common carotid artery (CCa); Subclavian Artery (SCa).



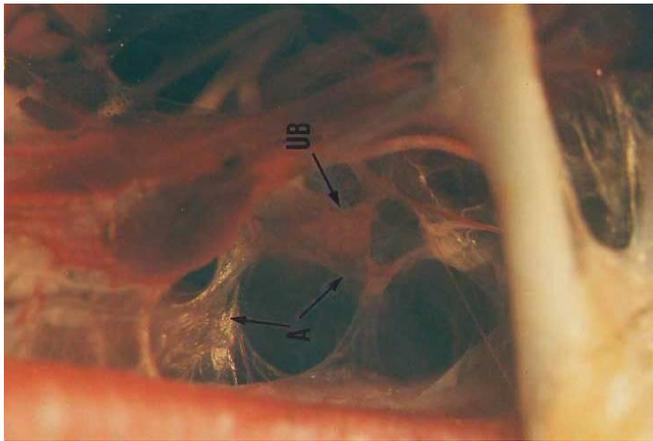
(Fig.2) Photograph show the shape and size of ultimobranchial in young gees. Left (LUB) and right (RUB). Insert show the left (LUB) and right (RUB) in adult geese.



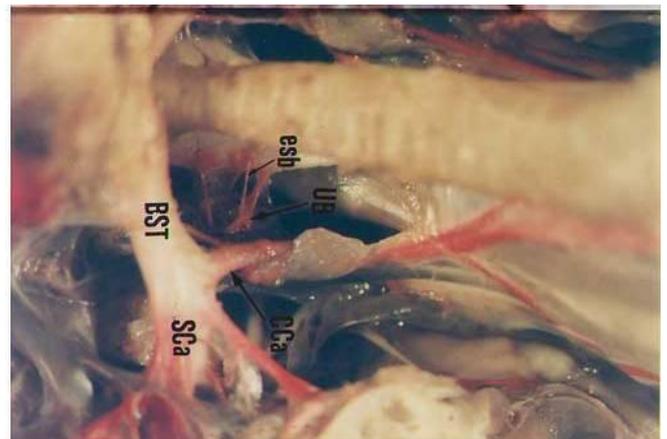
(Fig.4): Photograph shows the position of ultimobranchial gland in adult geese. Ultimobranchial gland (UB); Esophagus (es); Jugular vein (J); Vagus nerve (V).



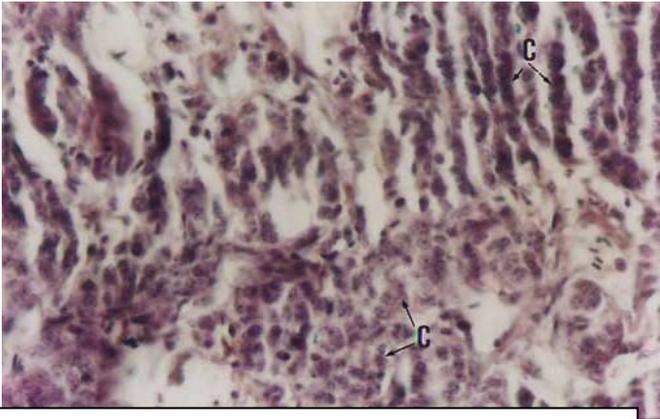
(Fig.5): Photograph shows the position of ultimobranchial gland in young geese. Left ultimobranchial (LUB); Right ultimobranchial (RUB); artery;(CCa). Parathyroid (P); Common carotid



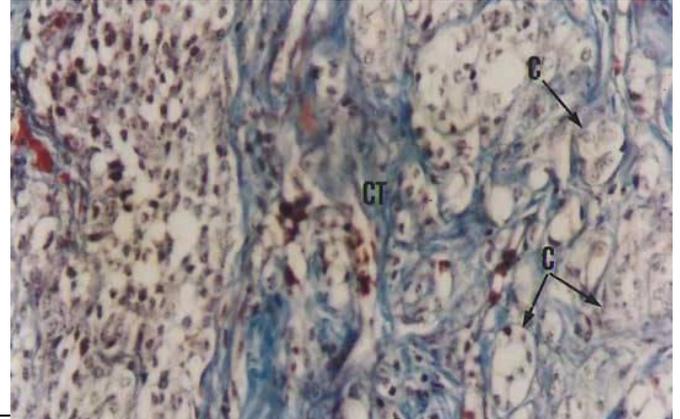
(Fig.6): Photograph shows the position of ultimobranchial gland in adult geese. Ultimobranchial gland (UB) is embedded in



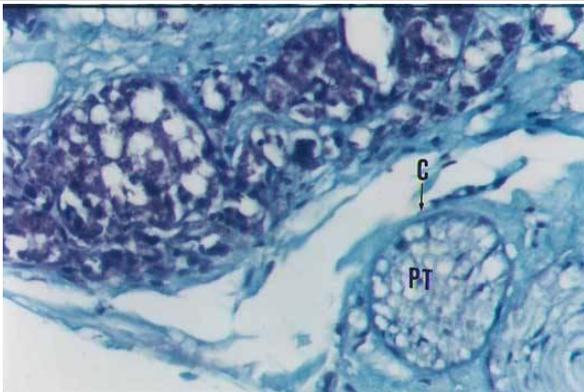
(Fig.7): Photograph shows the blood supply of ultimobranchial gland in adult geese. trunk Ultimobranchial gland (UB); Brachiocephalic Common carotid (BCT); Subclavian artery (SCa); branch (esb). artery; Esophagus



(Fig. 14): Photomicroscopic illustrates the cords of C-cells (C) have more aggregation of cells in the ultimobranchial gland of young geese.(H & E stain,40 X)



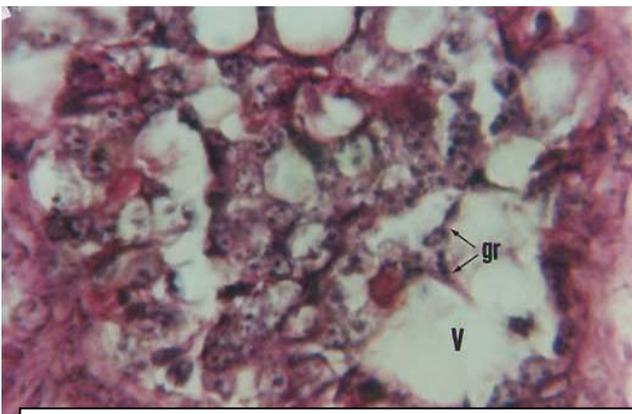
(Fig.13): Photo microscopic illustrates two types of C-cells in ultimobranchial gland of young geese, one type have light cytoplasm (L) and another cells have dark cytoplasm (D), also the secretion of these too.(H & E stain, 100 X). cells (se) is shown



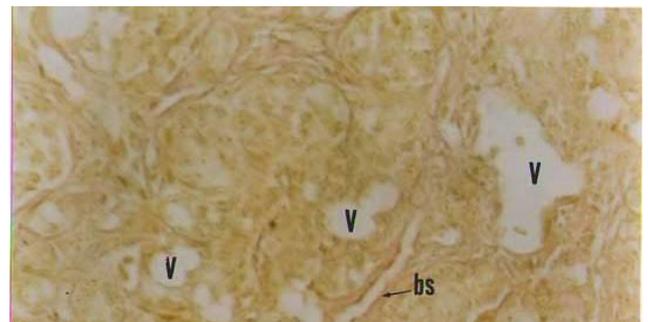
(Fig.16): Photo microscopic illustrates the accessory parathyroid nodule (PT) in ultimobranchial gland of adult geese enclosed by connective tissue capsule(C). (Masson trichrome stain, 40 X).



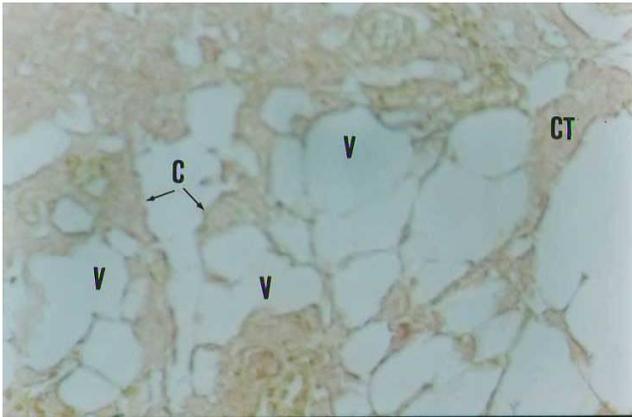
in (Fig.17): Photomicroscopic illustrates the stroma containing ultimobranchial gland of adult geese shapes and sizes vesicles (V) various in numbers, are empty and almost of these vesicles invaded by nerve fibers (N).(H&E stain , 40X)



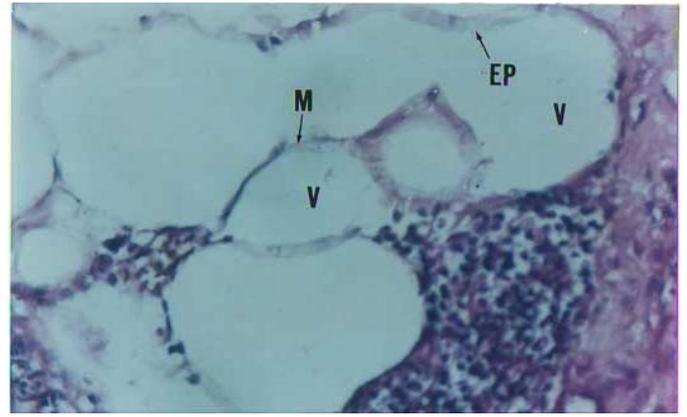
(Fig.18): Photo microscopic illustrates that granular vesicular lumens (V) have secretory material (gr) in ultimobranchial gland of adult geese. (PAS stain, 100 X)



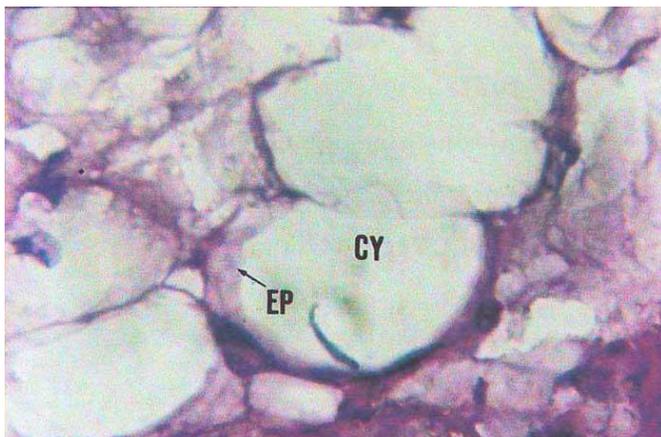
(Fig.19): Photomicroscopic illustrates the various of size of vesicles (V) in the ultimobranchial gland of young geese distributed among the stroma gland and these vesicles are invaded by blood Van Giesson stain, 40 X) vessels (bv).



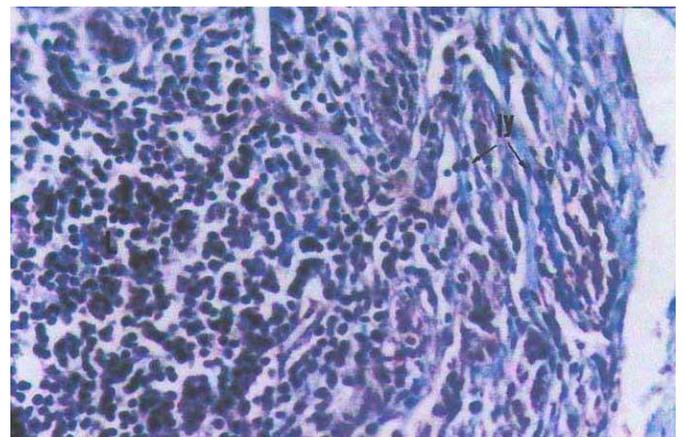
(Fig.20): Photo microscopic illustrates the various vesicles (V) in the ultimobranchial gland of adult geese surrounding by connective tissue stroma (CT). (Van –Giesson stain , 40 X)



(Fig.21): Photomicroscopic illustrates vesicles (V) of the ultimobranchial gland that are lined by simple cuboidal epithelium (EP) and this epithelium is very thin membrane (M), coalesce with each other. (H& E stain, 100 X)



(Fig.22): Photomicroscopic illustrates epithelial cells (EP) which lining the cyst (CY) in the ultimobranchial gland of adult geese and this cyst stores an staining fluid (H& E stain, 100 X)



(Fig.23): Photomicroscopic illustrates a single gland lymphoid foci (L) in ultimobranchial gland of young geese situated peripherally, scatter in the stroma of the gland. (Masson trichrome stain, 40 X).

Sex	Age	Weight of gland mg/1000g B.W.		B.W. $\mu \pm SE$
		Right gland $\mu \pm SE$	Left gland $\mu \pm SE$	
Female	Adult	A 2.74 \pm 0.34	B 5.82 \pm 0.72	3603 \pm 192.95
	Young	A 1.60 \pm 0.96	B 2.90 \pm 0.97	1093 \pm 51.15
Male	Adult	A 2.91 \pm 0.14	B 4.06 \pm 0.07	3080 \pm 136.57
	Young	A 1.27 \pm 0.17	B 2.560 \pm .28	975 \pm 43.30

Table (2) Diameter (mm) of the ultimobranchial gland in (adult , young) males and females geese

Sex	Age	Diameter of right gland $\mu \pm SE$	Diameter of left gland $\mu \pm SE$
Female	Adult	A 1.46 \pm 0.86	B 2.48 \pm 1.74
	Young	A 1.07 \pm 0.56	B 1.65 \pm 0.95
Male	Adult	A 1.72 \pm 1.07	B 2.50 \pm 0.14
	Young	A 0.84 \pm 0.79	B 1.73 \pm 1.05

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