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Microanatomy of the thyroid gland in sheep (*Ovis aries*)

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Abstract
A study on the Histomorphology and Histochemistry of the thyroid gland was conducted in the prenatal and postnatal age groups of sheep. For the prenatal study, the thyroid gland was collected from sheep embryos of early (42 days), mid (77.7 days) and late (128 days) gestation. Postnatal study included the collection of thyroid gland from young (<1 year), adult (1-5 years) and old (> 5 years) sheep, irrespective of the sexes. Tissue pieces were collected in different fixatives for routine paraffin embedding. Paraffin sections were used for routine and special staining techniques. Frozen sections were used for histochemical techniques. In the early gestation sheep embryo, only the central zone of the thyroid gland showed differentiated follicles. In the mid and late gestation embryos, the entire thyroid gland was differentiated into follicles. The location of C cells and ultimo branchia follicles was similar to the postnatal age groups. In the postnatal age groups, the thyroid gland was encircled by a connective tissue capsule. From the capsule, trabeculae entered the parenchyma and divided the gland into many lobules. The thyroid follicles varied in size and shape, in the different age groups studied. The diameter of the follicles showed a tendency to increase in size and became irregular in outline with advancing age. In the interfollicular area, in addition to the connective tissue fibres, nerves and capillaries, clusters of polyhedral cells were present. The ultimo branchia follicles were located in the perivascular connective tissue in the central zone and in the periphery of the thyroid gland within the capsule. The capsule of the thyroid gland was weakly PAS positive. The follicular cells and colloid showed strong PAS positive reaction. The accumulation of lipids and the presence of acid and alkaline phosphatases was noticed in the different structures of the thyroid gland.

Keywords: Histochemistry, histomorphology, prenatal, postnatal, sheep, thyroid gland

Introduction
The thyroid gland is a bilobed gland located just caudal to the larynx near the trachea in mammals. In some of the mammals, the two lobes are connected by an isthmus, across the ventral aspect of the trachea (Jones *et al.*, 1995) [36]. The histological appearance of the thyroid varies with function, age, sex, metabolic activity, breed, season of the year and geographic location (Jubb and Kennedy, 1970) [38]. The thyroid gland was first described anatomically in 1656 by Thomas Wharton (Barrington, 1975) [13] and Dickson (1977) [21] reported that the thyroid is one of the first endocrine glands to appear in the developing individual. Further, the thyroid is the only vertebrate alveolar endocrine organ in which the hormone is stored extracellularly within the gland (Gorbman and Bern, 1962) [24].

Follicles of ultimo branchia origin are common in avians and mammals and their location varies from animal to animal (Patt and Patt, 1969) [50]. In sheep, a residuum of the ultimo branchia body is frequently found in the thyroid gland, usually as ducts and these remnants may become cystic (Jubb and Kennedy, 1970) [38]. In chick and various mammals (sheep, pig, rabbit, man), thyroid function begins when one-third to one-half of the incubation or gestation period is completed, however in rat and mouse the thyroid function begins when 90 percent of the gestation is completed (Gorbman and Bern, 1962) [24]. In polybreeders like human being, the gland functions almost uniformly but in seasonal breeders, the gland is active at one stage and inactive at the other (Arora, 1992) [5].

Thyroid gland secretes thyroxine which governs the rate of metabolism and also influences the nervous system, growth (especially of bone) and the mental and physiological development of the organism. The activity of the thyroid is inter related with adrenal, the sex glands, mammary glands and thymus in mammals (Trautmannand Fiebiger, 1957; Jubb and Kennedy,

1970; Patt And Bloom and Fawcett, 1994) [67, 38, 16]. In addition, the thyroid hormones affect carbohydrate, protein and lipid metabolism (Cunningham, 1997) [18]. The parafollicular cells (C cells) of the thyroid gland produce thyrocalcitonin which lowers the blood calcium level by suppressing bone resorption (Mc Donald, 1989 and Dellmann, 1993) [45, 19].Thyroidectomy of immature sheep causes a decreased rate of weight gain and skeletal maturation, muscular weakness, decreased body temperature and heart rate, failure of reproductive maturation and a general lethargy. It also hampers the normal development of the wool-producing follicles leading to poor quality adult fleece, as wool production requires thyroxine in excess which is needed for growth (Dickson, 1977) [21].

Although an extensive work on the structure of the thyroid gland has been carried out in various mammals, the structural details of these glands in the sheep are limited. Hence, the present work has been undertaken with an aim to achieve the following objectives. i) To study the Histomorphology and histochemistry of the thyroid gland of the sheep in the prenatal and postnatal age groups. ii) To correlate the structures with the functional aspects of thyroid gland under light microscopy which would give further scope in the ultrastructural research of the thyroid gland in sheep.

Materials and Methods

Materials

The thyroid glands were collected from sheep for the present study. The materials were collected from the Chennai Corporation slaughter house, Perambur. For the prenatal study, the thyroid gland was collected from sheep embryos of early (42 days), mid (77.7 days) and late (128 days) gestation. Postnatal study included the collection of thyroid glands from young (<1 year), adult (1-5 years) and old (> 5 years) sheep, irrespective of the sexes.

The tissue pieces after washing with normal saline were fixed in the following fixatives for routine paraffin embedding (Luna, 1968) [42].

1. 10 percent buffered neutral formalin.
2. Formaldehyde solution (37-40 percent formalin).
3. Bouin's fluid.
4. Zenker's fluid.
5. Chilled formal calcium (4˚ C).

The fixed tissues were dehydrated in ascending grades of alcohol, cleared in xylene and embedded in paraffin (58˚ C - 60° C). 5-6 µm thin sections were cut and used for routine and special staining techniques.

For the localization of lipids and enzymes, 20-30 µm thick cryosections were used.

Methods

Histological staining methods

The following routine and special staining methods were used for histological observations.

1. Haemalum-Eosin phloxine method (Singh and Sulochana, 1996) for routine observations.
2. Masson's trichrome method (Luna, 1968) [42].
3. Van-Gieson's stain for collagen fibres (Singh and Sulochana, 1996) [65].
4. Weigert's method for elastic fibres (Luna, 1968) [42].
5. Gomori's method for reticulum (Luna, 1968) [42]. Mallory's phosphotungstic acid haematoxylin method (Luna, 1968) [42].
6. Bielschowsky's method for Axis cylinders and dendrites (Luna, 1968) [42].
7. Unna's method for mast cells (Luna, 1968) [42].
8. Mallory-Heidenhain Azan stain (Humason, 1962) [26].
9. Lead hematoxylin method (Humason, 1962) [26].

Histochemical staining methods

1. Periodic acid-schiff reaction for neutral mucopoly saccharides (Bancroft and Stevens, 1977).
2. Combined Alcian blue-PAS technique for acid and neutral mucopoly saccharides (Bancroft and Stevens, 1977).
3. Oil Red '0' in propylene glycol method for fats (Singh and Sulochana, 1996) [65].
4. Gomori's alkaline phosphatase cobalt method (Singh and Sulochana, 1996) [65].
5. Gomori's lead method for Acid phosphatase activity (Bancroft and Stevens, 1977)

Results

A. Histomorphology

1. Prenatal

1.1 Capsule and stroma

In the embryos of early (42 days), mid (77.7 days) and late (128 days) gestation, the capsule of the thyroid gland had three layers, similar to that of the postnatal age groups (Plate 1). The reticular fibres were morethan the collagen fibres in the capsule and stroma. The interfollicular connective tissue was sparse in the embryos of early and mid-gestation, but was comparatively more in the late gestation embryo. In all the stages of gestation, the interfollicular blood vessels surrounded each and every follicle in a basket like manner.

1.2 Parenchyma

In the embryos of early gestation (42 days), the peripheral area of the thyroid gland had the aggregation of follicular cells and a few follicles in the formative stages were observed. A few fully formed spherical follicles were also observed (Fig. 1). The centre of the gland was noticed with fully differentiated spherical thyroid follicles. In the mid (77.7 days) and late (128 days) gestation embryos, the entire thyroid gland was differentiated into thyroid follicles. In the early and mid-gestation embryos, only small and medium sized follicles were observed (Fig. 2). In the late gestation embryo, an apparent increase in the size of the thyroid follicles was observed throughout the parenchyma.

In the early and mid-gestation embryos, the thyroid follicles were lined by cuboidal cells with basophilic cytoplasm. In the late gestation embryo, the thyroid gland showed both active and inactive thyroid follicles. The active follicles were lined by tall cuboidal cells. The inactive ones were lined by low cuboidal cells. Numerous empty follicles were observed in the mid gestation embryo. The follicles with colloid were basophilic in the embryos in all the three stages of gestation. The colloid content was full in all the follicles in the late gestation embryo. The C cells were noticed in the intrafollicular and parafollicular locations in the embryos of all the three stages of gestation. The location and various forms of the ultimo branchia follicles in the prenatal age groups was similar to that in the postnatal age groups studied, except that stratification of the epithelium was not observed (Fig. 1, 2).

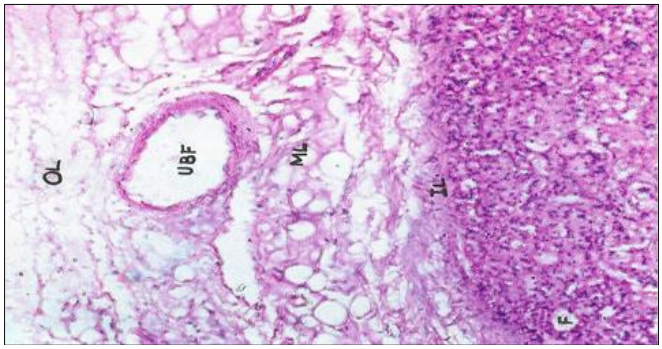

Fig 1: Photomicrograph of the thyroid gland of an early gestation (42 days) sheep embryo showing the three layers of capsule. O L-Outer layer; ML-Middle layer; IL-Inner layer; UBF-Ultimo branchia follicle; F-Follicle. H & E x 100

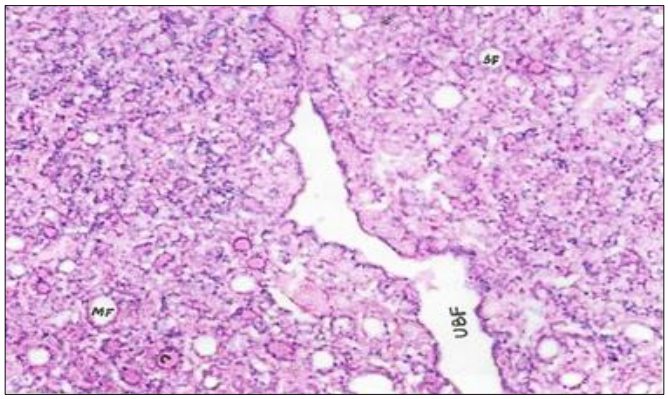

Fig 2: Photomicrograph of the thyroid gland of a mid-gestation (77.7 days) sheep embryo showing small and medium size follicles and a central narrow tubular ultimo branchial follicle. SF-Small follicle; MF-Medium follicle; UBF-Ultimo branchial follicle; C-Colloid. H & E x 200

2. Postnatal

2.1 Capsule and Stroma

In general, the thyroid gland was encapsulated by a collagenous connective tissue capsule, with few reticular fibres and a very few elastic fibres and fibroblasts in all the age groups studied. It was frequently thickened in areas, where small arteries, veins and nerves were present on the surface of the gland. The capsule had three layers (Fig. 3). The external layer was a mesothelial layer lined by simple squamous epithelium. The middle layer was rich in fat cells, blood vessels and nerves and also showed the presence of ultimo branchia follicles. The inner collagenous layer was closely adherent to the gland. The reticular fibres in the capsule were more in the thyroid of the young animals. In the older age groups, the collagen fibres predominated at the expense of the reticular fibres and a similar observation was also recorded in the isthmus of the thyroid glands.

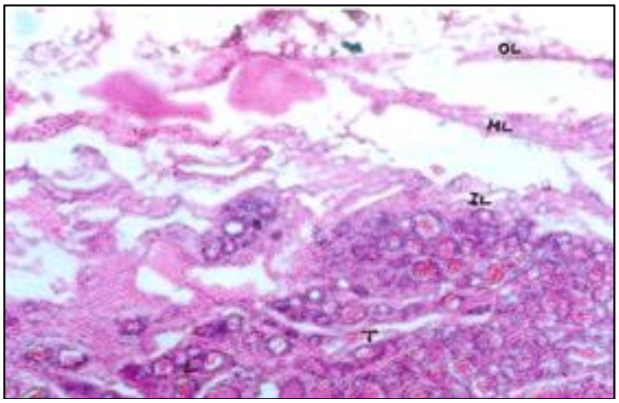

Fig 3: Photomicrograph of the thyroid gland of a 4-5 year old sheep showing the trabeculae and three layers in the capsule. OL-Outer layer; ML-Middle layer; IL-Inner layer; T-Trabeculae; L-Lobule. H & E x 100

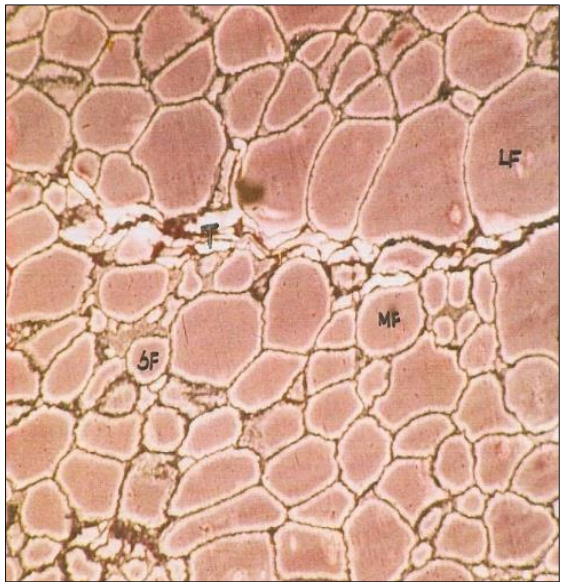

Fig 4: Photomicrograph of the thyroid gland of a <1 year-old sheep depicting the different shapes and sizes of the follicles.SF-Small follicles; MF-Medium follicle; LF-Large follicle; T-Trabeculae. Gomori's method for Reticulum x 100

From the capsule, trabeculae invaded into the parenchyma of the gland and formed lobules (Fig. 3). The collagen and reticular fibres predominated in the trabeculae (Fig. 4). The aggregation of thyroid follicles was observed within the lobules in all the age groups of the present study. The interfollicular connective tissue surrounded the follicles and formed a complete wrapping around each follicle in all the age groups, which increased as age advanced (Fig. 5). The capillaries and nerve fibres in the stroma surrounded each and every follicle in a basket like manner (Fig. 6). The follicular cells rested on a basement membrane.

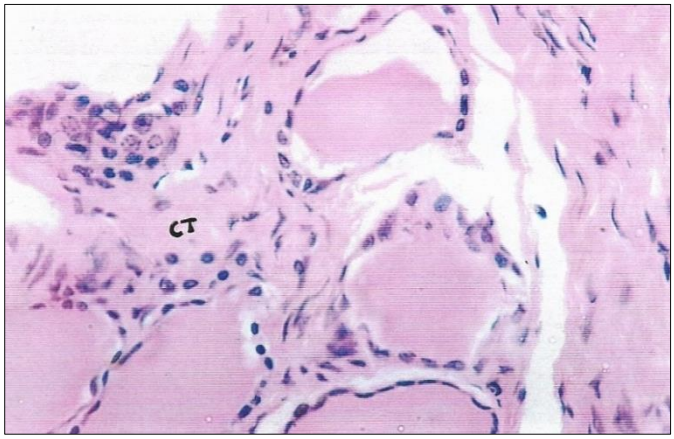

Fig 5: Photomicrograph of the thyroid gland of a 3 year old sheep showing the abundant inter-follicular connective tissue. CT-Connective tissue. H & E x 630

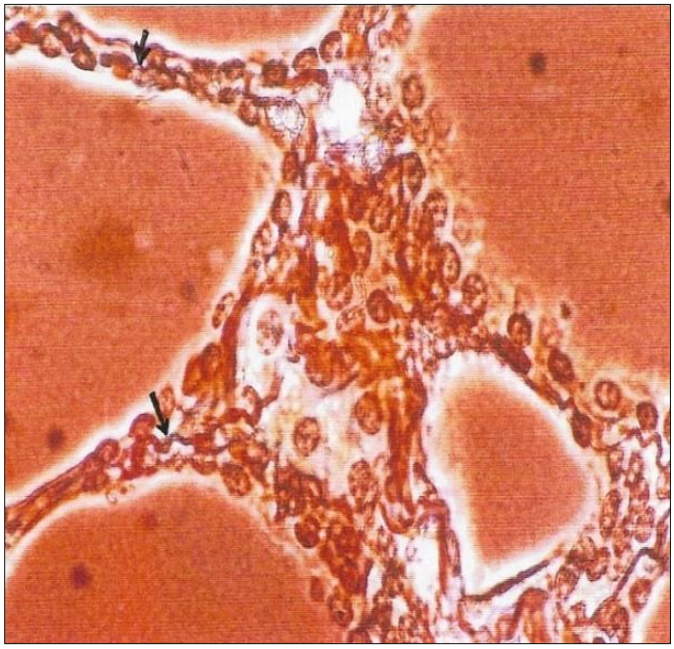

Fig 6: Photomicrograph of the thyroid gland of a 4-5 year old sheep showing the innervation of nerve fibres around the thyroid follicles.(arrows) Bielschowsky's x 100

ii. Parenchyma

a. Thyroid follicle

The thyroid follicles varied in size and shape. They were usually round to oval, but varying numbers of irregular, bilobed, polygonal and quadrilateral to pyriform follicles were also observed in all the age groups (Fig. 4). The active follicles were usually smaller when compared to the inactive follicles in all the age groups studied. However, the diameter of the follicles showed a tendency to increase in size and the follicles became irregular in outline with advancing age. The follicular epithelium consisted of two types of cells, namely, the follicular cells and the parafollicular or the light cells. The smaller active follicles were spherical in shape and were lined by cells varying from cuboidal to low columnar (Fig. 7). The cytoplasm was basophilic and finely granular in appearance. The nuclei were spherical to oval which appeared to be vesiculated with diffused chromatin material and occupied a greater proportion of the cell cytoplasm.

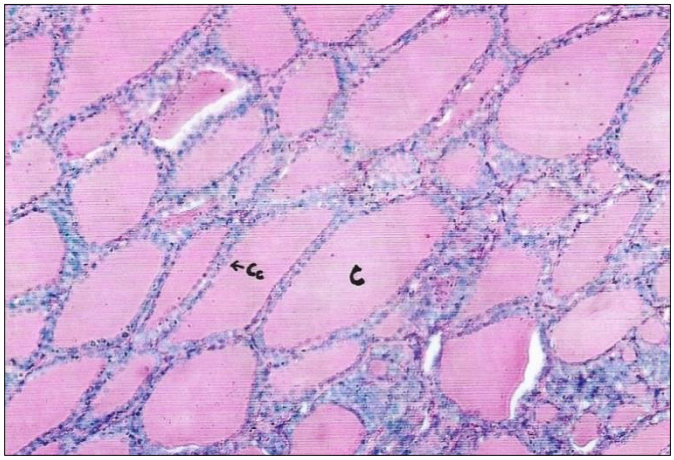

Fig 7: Photomicrograph of the thyroid gland of a 1 year- old sheep showing active follicles lined by cuboidal cells (arrow). H & E x 200

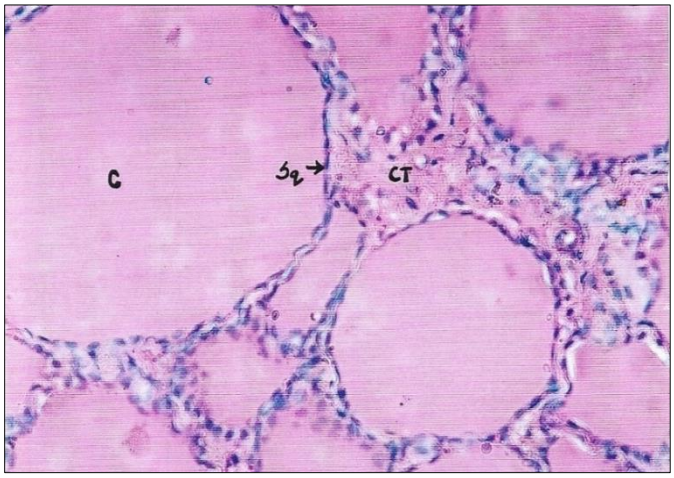

Fig 8: Photomicrograph of the thyroid gland of a >5 year-old sheep showing follicles lined by squamous epithelium (arrow) and the increased interfollicular connective tissue. Sq-Squamous cells; CT-Connective tissue; C-Colloid. H & E x 400

The larger inactive follicles were swollen with considerable amount of accumulation of colloid and the follicular epithelium was very low cuboidal to squamous (Fig. 8). The nuclei of the squamous cells were flattened, elongated and hyper chromatic. In all the age groups studied, the small and medium sized follicles dominated the larger follicles. The larger follicles were observed in the central zone of the thyroid gland, with only a few larger ones in the periphery. Empty follicles were more in the periphery of the thyroid than in the centre, in all the age groups studied (Fig. 9).Mitotic figures were observed in all the age groups. However, their occurrence decreased with progression of age and was rare in older age groups. Mast cells were observed in all the age groups studied. They were few in number in the interfollicular area and were more in number close to the blood vessels (Fig.10).In the interfollicular area, in addition to the connective tissue fibres, nerves and capillaries, clusters of polyhedral cells were present (Fig. 9). The finely granular cytoplasm of the polyhedral cells was faintly basophilic with round to oval nuclei. The histomorphological structure of the isthmus was similar to that of the thyroid except for the absence of C cells. The isthmus was glandular in the young animals, but showed a tendency to become fibrous with age.

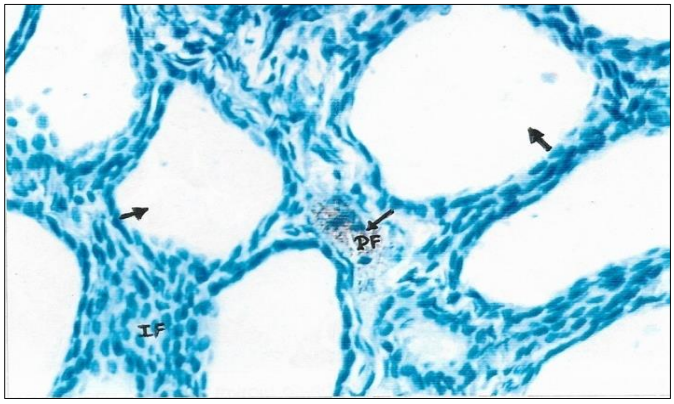

Fig 9: Photomicrograph of the thyroid gland of a 2 year-old sheep showing the localisation of parafollicular (C) cells in the parafollicular area (arrow) and the empty follicles (arrows). PF-Parafollicular cells; IF-Interfollicular cells. Lead hematoxylin x 630

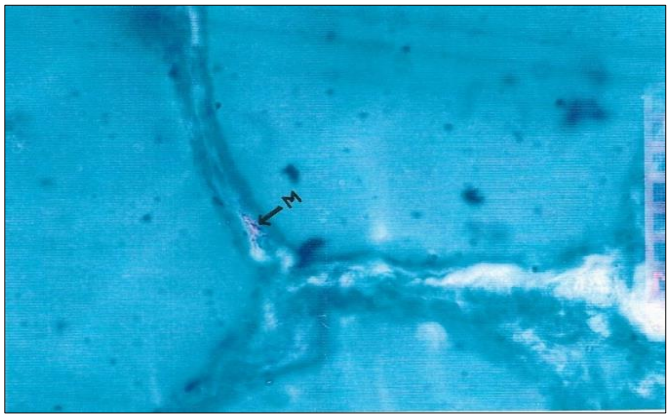

Fig 10: Photomicrograph of the thyroid gland of a >5 year-old sheep showing the presence of interfollicular mast cell (arrow). M- Mast cell. Unna's method x 1000

In the <1 year, 1 year and 2 year animals, the thyroid follicles were highly active and were lined by tall cuboidal cells. Numerous empty follicles were noticed in these age groups. In the 3 year and 4 -5 year animals, the apical borders of the active follicles were found to be disrupted with the release of colloid into the interfollicular space (Fig. 11).

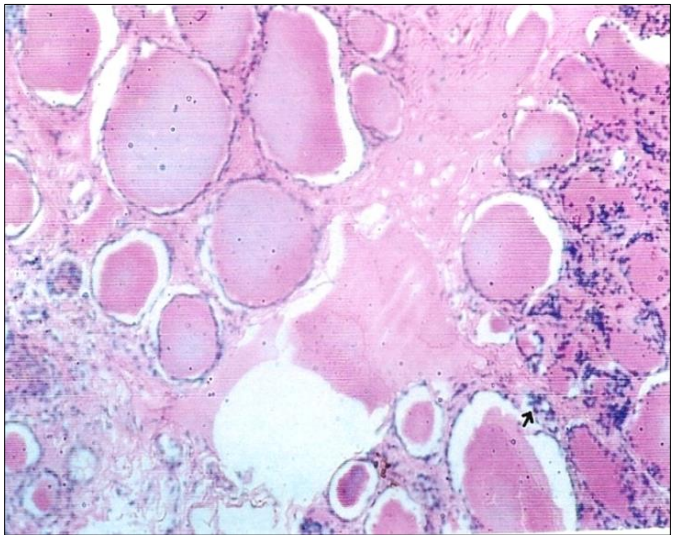

Fig 11: Photomicrograph of the thyroid gland of a 3 year old sheep depicting the rupture of the apical follicular membrane of a thyroid follicle (arrow). H & E x 200

In certain areas, the ruptured walls of the neighbouring follicles united to form bigger elliptical follicles.In the animal aged over 5 years, the inactive follicles were more compared to the other age groups. The luminal borders of the inactive follicles was intact and their colloidal content was in full (Fig. 8).

b. Thyroid colloid

The thyroid follicles were filled with a homogenous colloidal mass. In the follicles lined by simple cuboidal or low columnar epithelium, the colloid was predominantly basophilic whereas in the follicles lined by simple squamous epithelium, it was acidophilic in all the age groups. The quantity of the colloid contained in the follicle was variable. In the young animals, the colloid was less and homogenous whereas in the older animals, it was thick and of variable density. Over. Five years, the. Follicles was observed with colloid of very solid form. By Mallory-Heidenhain Azan stain, the colloid of the inactive follicles stained red while the colloid of the active Follicles appeared blue (Fig. 12).

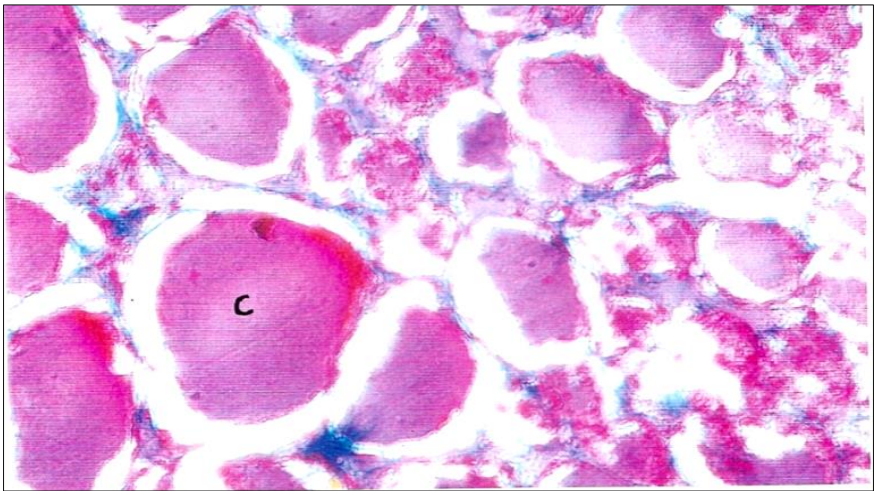

Fig 12: Photomicrograph of the thyroid gland of a 3 year old sheep showing the colloid in the follicles. C-Colloid. Mallory-Heindenhain Azan x 400

The presence of vacuoles was observed in the colloid of follicles in all the age groups. The vacuoles were of variable sizes and numbers and were seen abundantly at the periphery and occasionally in the central part of the colloid. The vacuoles were more in the peripheral follicles than in the central follicles, in all the age groups studied (Fig. 13).

Follicles with retracted colloid were observed in all the age groups. Many empty follicles were noticed in all the age groups except the age group of more than five years which had only a few empty follicles. The colloid cells of Langendorff were clumps or aggregation of desquamated • follicular epithelial cells. They were noticed in the inter-follicular spaces and within the thyroid follicles, in all the age groups studied but were more common in the older age group (Fig. 14).

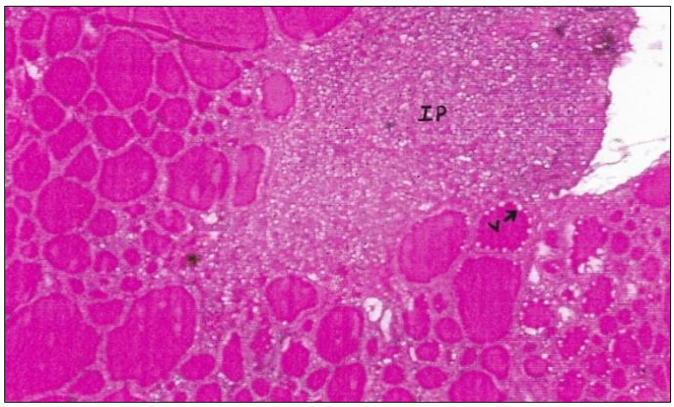

Fig 13: Photomicrograph of the thyroid gland of a < 1 year old sheep showing the internal parathyroid tissue intermingled with the thyroid follicles and the presence of peripheral vacuoles (arrow) in the colloid. IP-Internal parathyroid; V-Vacuoles. Combined Alcian blue-PAS x 100

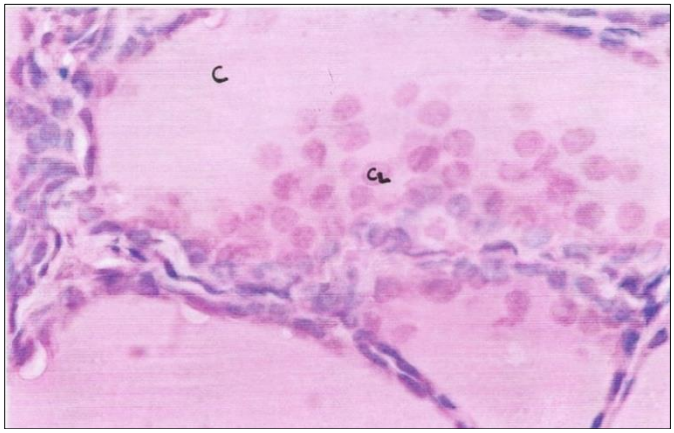

Fig 14: Photomicrograph of the thyroid gland of a > 5 year old sheep showing the colloid cells of Langendorff. C-Colloid; CL-Colloid cells of Langendorff. H & E x 1000

c. Parafollicular cells

The C cells were large with a spherical nucleus and a faintly stained cytoplasm. They were located mainly in the intrafollicular area (Fig. 15) and often in the parafollicular area (Fig. 9), but were never found touching the follicular lumen. The cytoplasmic granules of the C cells stained red with Mallory-Heidenhain Azan stain. With lead hematoxylin, the endocrine granules of C cells were stained black (Fig. 9).The C cells were not present in either the isthmus or in the Superior and inferior poles of the section of the thyroid. They were concentrated more in the deep central region of the lobes and decreased gradually towards the periphery. The number of C cells decreased as age advanced.

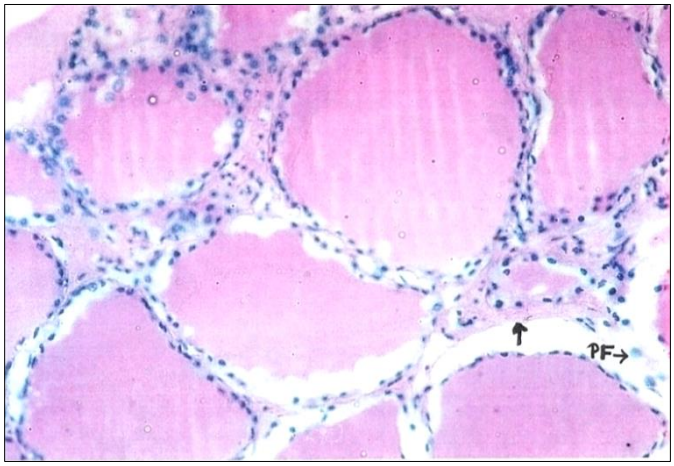

Fig 15: Photomicrograph of the thyroid gland of a 3 year old sheep showing the intrafollicular location (arrow) of parafollicular (C) cell (arrow). PF-Parafollicular cell. H & E x 400

d. Ultimo branchia follicles

The ultimo branchia follicles varied greatly in structure, shape, size and position in relation to the thyroid gland. They were located in the perivascular connective tissue, in the central zone of the thyroid gland and in the periphery of the thyroid gland, within the capsule. The inter-follicular connective tissue was more around the ultimo branchia follicles and formed a thick capsule with a few fat cells. Except in the older age group, the ultimo branchia follicles were numerous in all other age groups studied. Different shapes of the follicles namely, narrow and wide tubular, epithelial pearl like and bilobed ultimo branchia follicles were noticed. In the older animals, only one or two small tubular ultimo branchia follicles were observed in the periphery of the thyroid gland. The lumen of these follicles was filled with nucleated, non-nucleated and colloid debris and desquamation of the luminal epithelium was a common feature observed in all theage groups. The smaller ultimo branchia follicles were observed to be more active than the larger ones.

In the < 1 year and 4-5 year animals, a long branched tubular duct like ultimo branchia follicle, lined by stratified squamous epithelium, containing colloid in its lumen was noticed in the periphery of the thyroid gland, just beneath the capsule (Fig. 16). One branch of the tubular follicle extended along the periphery, while the other branch appeared penetrating the thyroid gland from outside and extended from the capsular connective tissue towards the centre of the gland. Close to the branches of the tubular ultimo branchia follicle, numerous colloid filled small thyroid follicles were observed which were lined by a cuboidal epithelium and were highly active. Retracted colloid was observed in some of the thyroid follicles, while the colloid of many follicles showed peripheral vacuoles. A few empty follicles were also noticed. Newly formed thyroid follicles just separated off from the ultimo branchia follicles were also seen lying close to its epithelial wall.

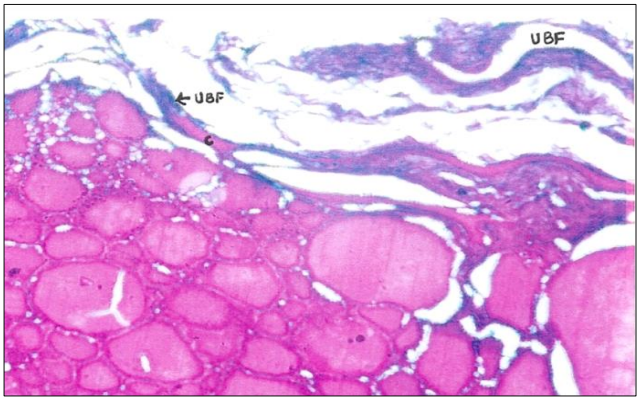

Fig 16: Photomicrograph of the thyroid gland of a 4-5 year old sheep showing branched tubular ultimo branchia follicle with PAS positive colloid in its lumen. UBF-Ultimo branchia follicle; C-Colloid. Combined Alcian blue-PAS x 100

In the < 1 year, 1 year and 2 year animals, and a small epithelial pearl like ultimo branchia follicle (Fig. 17) and in the 3 year animal, a large spherical ultimo branchia follicle was noticed in the perivascular connective tissue at the centre of the thyroid gland. The epithelial lining varied from stratified squamous to stratified cuboidal epithelium. The nuclei of the former type of epithelium were elongated and hyperchromatic, while the nuclei of the latter type were spherical with diffused chromatin material. The lumen of the ultimo branchia follicles contained a colloid like homogenous mass and desquamated cells. A few small thyroid follicles within the epithelial wall of the ultimo branchia follicles associated with the stratified epithelium was observed (Fig. 17). At certain locations, the epithelium of the ultimo branchia follicle showed discontinuity which was noticed, where there was an aggregation of epithelial cells rich in light cells (Fig. 18).

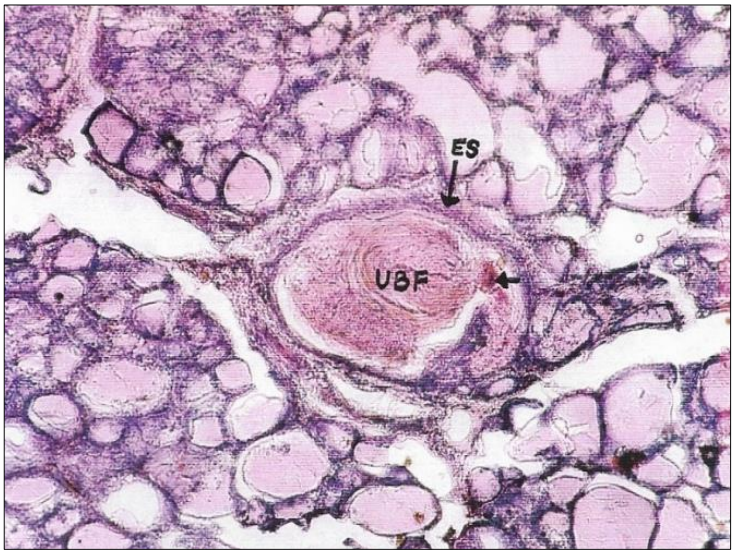

Fig 17: Photomicrograph of the thyroid gland of a 2 year old sheep showing the presence of epithelial stratification and lipids (arrows) in the ultimo branchia follicle. UBF-Ultimo branchia follicle; ES-Epithelial stratification. Oil Red 'O' x 100

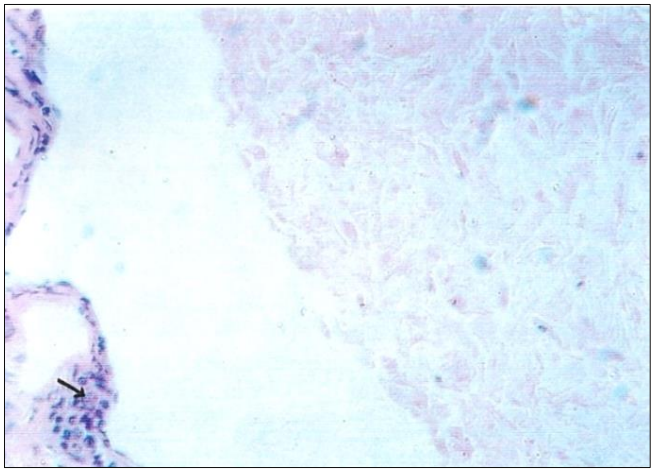

Fig 18: Photomicrograph of the thyroid gland of a 3 year old sheep depicting the association of C cells (arrow) in the epithelial lining of the ultimo branchia follicle.
H& E x 400

B. Histochemistry

In all the age groups, the capsule of the thyroid gland was weakly positive for PAS reaction. The follicular cells showed a strongly PAS positive supranuclear zone (Fig. 19). The infranuclear zone showed a moderate reaction for PAS. The follicular colloid was strongly positive for PAS reaction. The Parafollicular cells were negative for PAS reaction (Fig. 19). The colloidal material in the lumen of the ultimo branchia follicle was PAS positive (Fig. 16).The follicular cells, its colloid, the parafollicular cells and the colloid in the ultimo branchia follicle were negative for Alcian blue reaction in the combined Alcian blue-PAS technique followed.

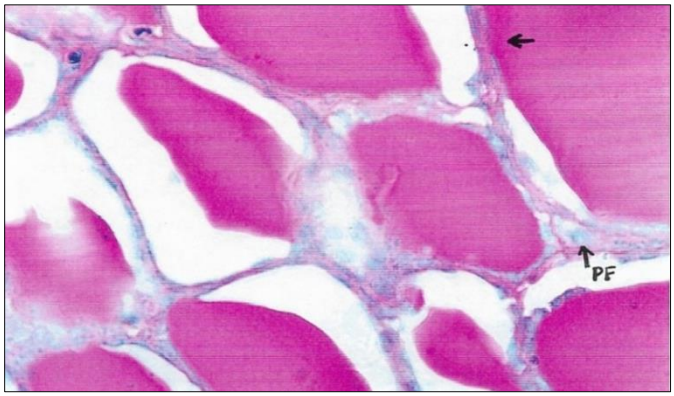

Fig 19: Photomicrograph of the thyroid gland of a 4-5 year old sheep showing the PAS positive supranuclear zone of the follicular cells (arrow) and the PAS negative parafollicular cell (arrow). PF-Parafollicular cell. PAS x 630

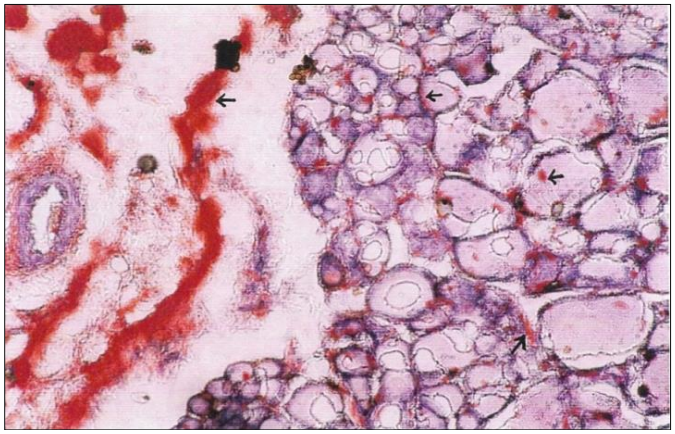

Fig 20: Photomicrograph of the thyroid gland of a 2 year old sheep showing the accumulation of lipids in the capsule, follicular cells, interfollicular connective tissue and the colloid (arrows). Oil Red 'O' X 100

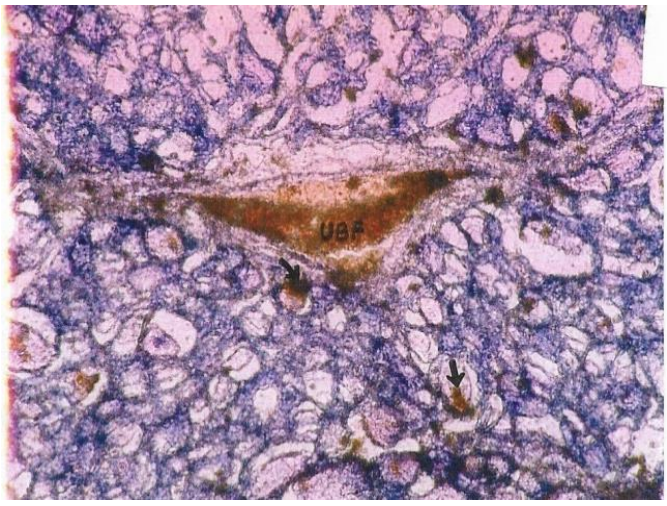

Fig 21: Photomicrograph of the thyroid gland of a 2 year old sheep showing acid phosphatase activity in the ultimo branchia follicle, interfollicular capillaries and the follicular cells (arrows). UBF-Ultimo branchia follicle. Gomori's lead method x 100.

The accumulation of lipids was observed in the capsule, follicular cells and its colloid, parafollicular cells, inter-follicular connective tissue and in the colloid of the ultimo branchia lfollicles (Fig. 20) and the lipid content increased as age advanced. Acid phosphatase activity was observed in the ultimo bronchial follicles, interfollicular capillaries and was moderate in the follicular cells at their luminal end in all the age groups (Fig. 21). Alkaline phosphatase activity was observed in the capillaries and in the epithelium of the ultimo branchial follicles.

Discussion

A. Histomorphology

1. Prenatal

In the thyroid glands of sheep embryos of early (42) days, mid (77.7) days and late (128) days of gestation, the capsule had three layers similar to the postnatal age groups. In all stages of gestation, the interfollicular blood vessels surrounded each and every follicle in a basket like manner as reported by Balasundaram (1995) in chicken embryos. In the early and mid gestation embryos, small and medium sized spherical follicles lined by cuboidal epithelium were observed. This is in accordance with the findings of Mathur (1971) [44] in buffalo fetuses. Follicles with colloid were observed in the early gestation embryo in the present study which concurs with the findings of Igbokwe (2013) [30] who observed colloid in the foetal thyroids of goats at 30 days of gestation. Contrary to this, Alwan (2009) [4] reported the follicular development with colloid in sheep at 75 days of gestation. Bello *et al.* (2014) [14] observed follicular organisation in camel foetuses at the 2nd trimester of gestation. Further, the presence of colloid was reported in prenatal cattle by Schafie and Mashaly (1974) [64]. Ranjan *et al*. (2011) [54] observed the appearance of follicular arrangement in the developing thyroid of buffalo foetuses at 119 days of gestation with a strongly PAS positive colloid in some of the follicles. Ramayya *et al.*(2012) [53] reported that in buffalo foetuses, at 115 days of gestation the thyroid primordium showed formation of follicles and that at 143 days, characteristic thyroid follicles lined by cuboidal epithelium with a distinct colloid substance was also observed. El Sheik *et al*. (1966) [22] observed follicular development which appeared at 52 days of gestation in camel. The colloid content was full in all follicles in the late gestation embryo in the present study which concurs with the findings of Igbokwe and Ezeasor (2015) [31] who observed colloid in the late foetal stage follicles (85-110 days) in large white crossbred pigs. Rocha *et al* (2010) [55] had observed that human fetus with 23 weeks of age did not show any sign of follicles or colloid and the one with 35 weeks of age presented follicles with colloid. In contrast, Gaikwad *et al*. (2012) [23] had detected a PAS-positive colloid in the human fetal thyroid at 13th -14th week of gestation. In the present study, the C cells were located in the intrafollicular and parafollicular positions in the early (42 days) gestation embryo, which is in corroboration with the findings of Jordan *et al.* (1973) [37] who has recorded the C cells in 33 days sheep embryo and Igbokwe and Ezeasor (2015) [31] who observed large parafollicular cells in the early foetal pig thyroid. Igbokwe *et al.* (2014) [32] reported that C cells were apparent in a parafollicular position at 150-210 days foetuses of white Fulani (Zebu) cattle. Ranjan *et al*. (2011) [54] observed C cells in buffalo foetuses at 220 days of gestation. The location and various forms of the ultimo branchia follicles observed in the prenatal age groups was similar to that in the postnatal age groups studied, except that stratification of the epithelium was not observed. This indicates that these ultimo branchia follicles could be of the precursor forms, which is in total agreement with the findings of Wollman and Neve (1971) [69] in rat embryos. However, ultimo branchia follicles lined with stratified epithelium were observed in the developing thyroid of buffalo foetuses at 130-137 days of gestation by Ranjan *et al*.(2011) [54]. Sayed *et al.* (2005) [63] had also observed ultimo branchia remnants in the thyroid gland of buffalo.

2. Postnatal

i. Capsule and stroma

The thyroid gland of the sheep was encapsulated by a collagenous connective tissue, with few reticular fibres and a very few elastic fibres as reported by Roy *et al.* (1978 b) [59] in goat and Ashok *et al.* (1993) [6] in the small and large ruminants. The capsule had three layers from without inwards namely, the mesothelial layer, the vascular layer and the fibrous layer as reported by Adhikary *et al.* (2003) in black Bengal goat, Yaswant Singh and Bharadwaj (1982) [70] and Balasundaram (1995) in white leghorn chicken. The reticular fibres in the capsule were more in the thyroid of young animals. In older age groups, the collagen fibres predominated at the expense of reticular fibres and a similar observation was also recorded in the isthmus of the thyroid glands. This is in agreement with the findings of Roy *et al.* (1978 b) [59] in

goat.From the capsule, connective tissue trabeculae radiated and divided the gland into many lobules each containing number of follicles. This is in accordance with the findings of Roy *et al.* (1978 b) [59] in goat, Bacha and Wood (1990) in cow and pig, Dellmann (1993) [19] in mammals and Aughey and Frye (2001) [8] in animals.

In the present study, the capillaries and nerve fibres in the stroma surrounded each and every follicle in a basket like manner. It might be a provision for easy emptying of follicular secretion into the blood stream. The interfollicular connective tissue formed a complete wrapping around each follicle. This is in accordance with the findings of Roy *et al.* (1978 b) [59] in goat and Ahmed *et al.* (2016) in dromedary camels. Contrary to this, Isler *et al.* (1968) [35] described an incomplete wrapping of follicles by connective tissue in cattle, horse, dog, pig, monkey, rat, rabbit, guinea pig and hamster.

ii. Parenchyma

a. Thyroid follicle

As reported by Ashok *et al.* (1993) [6] in small and large ruminants, the follicular cells rested on a basement membrane in the present study. The thyroid follicles varied in size and shape. The diameter of the follicles showed a tendency to increase in size and the follicles became irregular in outline with advancing age. This is in agreement with the observations made by Mathur (1971) [44] in buffaloes, Roy *et al.* (1978 b) [59] in goats, Adhikary *et al.* (2003) in black Bengal goats, Kausar and Shahid (2006) [39] in one-humped camel and Igbokwe and Ezeasor (2015) [31] in white cross bred pigs. This diversity in the size and shape of the follicles might be related to the functional status of the follicles at that time.

The follicles were lined by a single layer of epithelium, immaterial of the size and shape of the follicle and the age of the animal. Majority of the follicles were lined by a cuboidal epithelium excepting those inactive follicles, where the lining epithelium turned into a squamous epithelium. However in the highly active follicles, invariably it was a columnar epithelium. These observations are similar to the findings of Roy *et al.* (1978 b) [59] in goats, Ashok *et al.* (1993) [6] in small and large ruminants, Dellmann (1993) [19] and Banks (1993) [12] in animals, Di Fiore (1989) in humans,Yaswant Singh and Bharadwaj (1982) [70] in birds, Abdel-Magied (2000) in camel, Kausar and Shahid (2006) [39] in camel, Ali *et al.* (2015) [2] in Iraqian sheep, Shehan (2017) [46] in Iraqian goats and Khaleel and Salih (2017) [41] in sheep.

The above histomorphological structural variations in the follicular epithelium might be as a result of the mechanical pressure on the epithelium caused by accumulation of the colloid distending the follicles, or by the altered density of the basement membrane and by the increase in the collagen fibres, which act as a barrier in the exchange between the plasma and the epithelium. Diversified physiological statuses of the gland could also be responsible for these changes of the follicular epithelium as opined by Roy *et al.* (1978 b) [59] in goats.In the active follicles, the apical borders of the follicular cells were found to be lost with signs of release of colloid, suggesting an apocrine mode of secretion in the follicular cells of the thyroid in sheep which concurs with the findings of Roy *et al.* (1978 b) [59] in goats, Atoji *et al.* (1999) [7] in camels and Sathyamoorthy (1996) [62] in Japanese quails. Small and medium sized follicles dominated over the larger ones, which is in accordance with the findings of Yaswant Singh and Bharadwaj (1982) [70] and Balasundaram (1995) in white leghorn chicken.

Roy and Yadava (1977) [56] in buffaloes, Baishya *et al. (1985)* [10] in kid, Adhikary *et al.* (2003) in black Bengal goats, Sanap *et al.*(1998) [61] in Cattle and Altaay (2007) [3] in Iraqi buffalo described the larger follicles in the central zone of the thyroid, which concurred with the present findings. However this is in contrary to the findings of Tsuchiya *et al.* (1984) [68] in pigs, Maiti (1980) [43] in rat,Ashok *et al.* (1993) [6] in small and large ruminants, Khaleel and Salih (2017) [41] in sheep and Ahmadpanahi and Yousefi (2012) in one humped camel, who observed smaller active follicles in the centre and larger inactive follicles in the periphery of the gland.The occurrence of mitotic figures decreased with the progression of age and was rare in the older age groups, as reported by Roy *et al.* (1978 b) [59] in goats. Clusters of polyhedral cells which resembled the follicular cells were present in the interfollicular area. These cell clusters were the tangential sections of the thyroid follicle through the follicular wall. This is in accordance with the findings of Roy and Yadava (1977) [56] in buffaloes and DiFlore (1989) in humans.

b. Thyroid colloid

The thyroid follicles were filled with a homogeneous colloidal mass. In the follicles lined by simple cuboidal or low columnar epithelium, the colloid was predominantly basophilic, while in those follicles lined by simple squamous epithelium it was acidophilic. This is in agreement with the findings of Banks (1993) [12] in animals and Yaswant Singh and Bharadwaj (1982) [70] in white leghorn birds. Beresford (1983) [15] recorded that the thyroid colloid was acidophilic when dense and basophilic when dilute. The present study also confirms the earlier findings. By Heidenhain Azan stain, the colloid of the inactive follicles stained red while the colloid of active follicles stained blue. The nature of the colloid varied according to the activity of the thyroid gland. In the inactive follicles, it was more and thick due to the accumulation of large amount of colloid without being utilized, whereas in active follicles it was lesser and thinner due to regular production and consumption. This is in agreement whit the finding of Adhikary *et al.* (2003) in black Bengal goats. The solid form of colloid observed in the older age groups might be due to prolonged stasis of the colloid in the follicle. These findings are similar to the views put forth by Roy *et al.* (1978 b) [59] in goats.

The presence of peripheral vacuoles in the colloid of active follicles is similar to the observations made by Bacha and Wood (1990) and Dellmann (1993) [19] in animals, Ashok *et al.* (1993) [6] in the small and large ruminants, Peksa *et al.*(2011) [51] in Slaughter cattle, Ahmed(2016) in dromedary camels, Igbokwe and Ezeasor(2015) [31] in white crossbred pigs, Khaleel and Salih (2017) [41] in sheep, DiFlore (1989) in humans and Yaswant Singh and Bharadwaj (1982) [70] in white leghorn birds. From the appearance of vacuoles at the periphery of the colloid, it is felt that the elimination process of the secretory material begins from the outer limitation of the colloidal mass. This concurs with the earlier reports of Bloom and Fawcett (1994) in humans. The colloid cells of Langendorff were observed in many of the follicles in the present study. A similar observation was made by Adikary *et al.* (2003) in black Bengal goats. These cells are believed to be the aged desquamated cells of the follicles which give way for the new developing cells in the thyroid gland of human beings as stated by Kelly *et al.* (1984) [40].

c. Parafollicular cells

The C cells were large with a spherical nucleus and a faintly stained cytoplasm. They were found singly or in small groups, located mainly in the intrafollicular and often in the parafollicular area. This is in accordance with the findings of Okada *et al.* (1990) [49] in sheep, Roy *et al.* (1983) [58] in caprine, Ashok *et al.* (1993) [6] in small and large ruminants, Roy and Yadava (1973) [56] in buffaloes, Bacha and Wood (1990) in animals, Yaswant Singh and Bharadwaj (1982) [70] in white leghorn birds and Khaleel and Salih (2017) [41] in sheep. Further, the parafollicular cells observed as single cell in the epithelial lining of the follicles in the present study is in agreement with the findings of Nouri and Babak (2010) in ewe, Ali *et al*.(2015) in Iraqian sheeps, Igbokwe *et al*. (2015) [31] in West African dwarf goats, Shehan(2017) [46] in Iraqian goats, Peksa *et al*.(2011) [51] in slaughter cattle and Swsen Ali (2014) in donkeys. C cells found in small groups in between the follicles in the present study coincides with the finding of Prasanth *et al*.(2012) in cats. The cytoplasmic granules of the C cells stained red with Mallory-Heidenhain Azan stain as reported by Roy *et al.* (1978 b) in goats. With lead hematoxylin the granules of C cells stained black. Hurtrel (1974) [27] identified C cells in cat by the lead hematoxylin method. Kelly *et al.* (1984) reported that the calcitonin cells might be often considered as part of the Amine Precursor Uptake and Decarboxylation System (APUD) in mammals.

The C cells were not present in either the isthmus or in the superior and inferior poles of the thyroid as reported by Okada *et al.* (1995) in sheep and Igwenagu*et al*. (2006) in camel.On the contrary Roy *et al.* (1983) [58] in goats, Khaleel and Salih (2017) [41] in sheep and Hussin and Altaay (2009) [28] in buffalo observed the presence of C cells in the isthmus of the thyroid gland. The C cells were concentrated more in the deep central region of the thyroid lobes as stated by Okada *et al.* (1995) in sheep, Nieto and Fdez de Troconiz (1988) [47] in sheep, calf, horse, dog and pig and Tsuchiya *et al.* (1984) [68] in pigs. The number of C cells decreased as age advanced in the present study, which concurs with the findings of Hurtrel (1974) [27] in cat and Balasundaram (1995) in white leghorn birds. On the contrary, Zabel (1987) [71] in rats and Igbokwe and Ezeasor (2015) [31] in white crossbred pigs observed that the C cells increased in number with age.

d. Ultimo branchia follicles

The ultimo branchia follicles of various forms and locations in relation to the thyroid glands were observed in the different age groups of sheep studied. Similar findings were reported by Roy *et al.* (1978 a) in goats and Roy and Saigal (1986) [57] in sheep. Wolman and Neve (1971) [69] noticed ultimo branchia follicles in Fischer rat, in all the thyroid lobes studied. Three forms of the ultimo branchia follicles namely narrow and wide tubular, epithelial pearl like, bilobed and a group of ultimo branchia follicles were observed in different age groups of the present study. The presence of bilobed and a group of ultimo branchia follicles were considered to be the result of branching of the main stem, because of their appearance in adjacent sections. Though this is in accordance with the findings of Roy *et al.* (1978 a) who reported similar forms of ultimo branchia follicles in goats, he has also mentioned two other forms namely, a long follicle with colloid and a sheet of stratified squamous cells attached to the ultimo branchia follicle. The ultimo branchia follicles observed in the present study were found to be located in the periphery of the thyroid gland within the capsule and in the perivascular connective tissue in the central zone of the thyroid gland as reported by Roy *et al.* (1978 a) in goats.

The ultimo branchia follicles in the present investigation were found to be lined by a stratified epithelium, which varied from stratified squamous to cuboidal type. The stratification of epithelial cells has been considered as a criterion to distinguish the ultimo branchia follicles from the thyroid tissue (Gorbman and Bern, 1962) [24]. Further, stratification and desquamation distinguished the matured ones from its other forms namely the precursor and intermediate types in rat (Wollman and Neve, 1971) [69].The presence of C cells along the ultimo branchia follicular epithelium has been observed in the present study which concurs with the findings of Roy *et al. (*1978 a) in goats and Roy and Saigal (1986) [57] in sheep. It could be presumed that, though C cells originated from the ultimo branchia follicles and got incorporated with the thyroid follicles in the embryonic stages, the ultimo branchia follicles continued to contribute these cells even after birth. Similar views have been putforthby Roy *et al.* (1978 a) in goat and Calvert (1972) [17] in rat.

The present study also revealed that the ultimo branchia follicular epithelium was associated with the follicular cells of the thyroid and in many instances; follicular cells pinched off from it and formed small thyroid follicles. By such an act the ultimo branchia follicles appeared to be a source of thyroid follicular cells, as reported by Roy *et al.* (1978 a) in goats and Roy and Saigal (1986) [57] in sheep. Harach (1986) [25] observed solid cell nests in the adult human thyroids, which were the ultimo branchia vestiges and served as a source of thyroid follicular cells and C cells.The lumen of the ultimo branchia follicles contained nucleated and non-nucleated colloid debris and desquamated epithelial cells as reported by Roy *et al.* (1978 a) in goats, Roy and Saigal (1986) [57] in sheep and Wollman and Neve (1971) [69] in rats.

B. Histochemistry

The capsule of the thyroid gland was weakly positive for PAS in the Combined Alcian blue-PAS reaction. It shows that the capsule was mainly made up of neutral mucopolysaccharides. The follicular cells were PAS positive as reported by Roy and Saigal (1987) in sheep, Ali *et al* (2015) [2] in Iraqian sheeps, Shehan(2017) [46] in Iraqian goats,Roy and Yadava (1977) [56] in buffaloes and Yaswant Singh and Bharadwaj (1982) [70] in white leghorn birds. The positive reaction of follicular cells for acid muco polysaccharides observed by Roy and Saigal (1987) in sheep was not found in the present study. The C cells were negative for PAS and acid muco polysaccharides, which concurs with the findings of Roy and Saigal (1987) in sheep, Roy *et al.* (1983) [58] in goat and Roy and Yadava (1973) [56] in buffalo.

The follicular colloid was PAS positive as reported by Roy and Yadava (1977) [56] in buffalo Dellmann (1993) [19] in animals and Khaleel and Salih (2017) [41] in sheep. The colloidal material in the lumen of the ultimo branchia follicle was PAS positive as reported by Roy *et at* (1978 a) in goat and Roy and Saigal (1986) [57] in sheep. The positive reaction of the colloid in the ultimo branchia follicle for the acid muco polysaccharides observed by Roy and Saigal (1986) [57] in sheep was not observed in the present study. The accumulation of lipids was observed in the follicular cells, C cells, interfollicular connective tissue, and colloid of the follicular cells and in the ultimo branchia follicles which concurs with the findings of Roy and Saigal (1986, 1987) [57] in sheep. The presence of lipids in the follicles and connective

tissue was reported by Ali *et al.* (2015) [2] in Iraqian sheep and, Shehan (2017) [46] in Iraqian goats. In addition, the capsule also showed the presence of lipids in the present study. Acid phosphatase activity was observed in the interfollicular capillaries and was moderate in the follicular cells at their luminal end as reported by Roy and Saigal (1987) in sheep and Roy *et al.* (1993) in donkeys. In addition the present study showed acid phosphatase activity in the ultimo branchia follicle also. Alkaline phosphatase activity was observed in the capillaries and in the epithelium of the ultimo branchia follicles as reported by Roy and Saigal (1987) in sheep. Further Roy *et al.* (1993) reported alkaline phosphatase activity in the stromal capillaries in donkeys.

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