HISTOLOGICAL AND HISTOCHEMICAL STUDIES ON THE THYROID GLANDS OF INDIAN BUFFALO (BOS-BUBALIS)

By

Kripa Shankar Roy, B. V. Sc. & A. H.
Rajendra Agricultural University
Junior Fellow in Anatomy.

A THESIS

submitted to the Faculty of Veterinary Science,
Rajendra Agricultural University, Patna (Bihar)
in partial fulfilment of the requirements
for the degree of
MASTER OF SCIENCE (Veterinary).

Post-graduate Department of
ANATOMY.

BIHAR VETERINARY COLLEGE, PATNA.
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Dated, the 10th March, 1972.

Certified that this thesis entitled "Histological and Histochemical studies on thyroid glands of Indian buffalo (Bos bubalis)" has been prepared under my supervision and guidance by Shri Kripa Shankar Roy, an Rajendra Agricultural University Fellow in Anatomy. I have checked his observation and results from time to time during the period of this study. The thesis has been submitted to Rajendra Agricultural University, Bihar, Patna, for the degree of MASTER OF SCIENCE (VETERINARY) and it incorporates the results of his independent studies.

(R.C.P. Yadava)

I am extremely grateful to the "Rajendra Agricultural University, Bihar, Patna for award of a Junior Fellowship in shape of financial assistance for undertaking this project.

(AUTHOR)

ABSTRACT

Histological and Histochemical studies on the thyroid gland of 10 young male and 10 old female buffaloes were made. Haematoxylin and eosin was used as routine stain. Other special stains were employed to reveal the histological and histochemical details.

The average length, breadth, thickness and volume of the left lobe in young animal were 2.55 cm., 1.66 cm., 0.70 cm. and 2.44 ml. respectively, while that of the adult animals were 4.52 cm., 3.61 cm., 1.19 cm. and 11.30 ml. respectively. The average length, breadth, thickness and volume of right lobe of young animal were 2.06 cm., 1.66 cm., 0.84 cm. and 1.73 ml.respectively while that of adult animal were 3.82 cm., 3.45 cm., 1.17 cm. and 8.10 ml. The total weight of whole gland of young and adult were 4.20 gm. and 21.72 gm. The left lobe was found to be larger than right lobe. Absence of Isthmus was observed in one female buffalo. Its length, breadth and volume in young animal were 3.63 cm., 0.24 cm. and 0.74 ml. respectively, while that of adult animals were 7.16 cm., 0.58 cm. and 1.70 ml.

The capsule of thyroid gland was arranged in three layer. The outer and inner layer was made up of fibrous connective tissue whereas the middle layer was of adipose tissue. The gland was made up of numerous follicle with small amount of stroma. In young experimental animals the diameter of the round follicle varied from 47.6 /u to 130.4 /u. In case of smallest oval follicle the minor diameter was 50.4 /u and major 89.0 /u. In largest oval follicle the minor diameter was 226.8 /u and major 322.0 /u.

In the adult animals the diameter of round follicle varied from 92.6 /u to 351.4 /u. In the smallest oval follicle the minor diameter was 114.2 /u and major 152.8 /u. In largest oval follicle the minor diameter was 354.2 /u and major 575.2 /u. The larger follicles appeared in deeper area of the gland. When the follicles were cut tangentially it appeared as a clump or aggregation of cells.

The follicles were lined either by simple squamous or simple cuboidal or simple columnar epithelium. They had basophilic cytoplasm and contained flattend elongated or oval or spherical nuclei. Nucleus was strongly feulgen reactive. Mitotic figures were found among the follicular cells. In young animals the average height, breadth of squamous follicular cell was found to be 4.13 /u and 7.85 /u, the cuboidal follicular cell was 6.43 /u and 6.55 /u and columnar follicular cell was 9.52 /u and 5.32 /u. The diameter of nucleus was 3.48 /u. In adult animals the height and breadth of squamous follicular cell was 4.30 /u and 8.06 /u, the cuboidal follicular cell was 6.75 /u and 6.86 /u and columnar follicular cell was 10.45 /u and 6.09 /u. The diameter of nucleus was 4.04 /u.

Light cells were seen among the follicular cell proper. They constituted the second epithelial component of the thyroid gland. They had large nucleus with faintly stained cytoplasm. They were devoid of uniform contour and had many shapes. The light cells were located among the droplet laden follicular cells or between the follicular cell and basement membrane. They did not contain colloid droplets. They were separated from direct contact with colloid by whole follicular cell or by a layer of its cytoplasm.

They showed argyrophilia when stained with Cajal stain and Weil Davenport stain. The cynophilic secretory granules of these cells stained blue or red by Mallory stain or Heidenhains azan stain.

In young animals the height, breadth of the light cell and diameter of nucleus was found to be 12.44 μ, 10.45 μ and 4.63 μ respectively whereas in the adult the height, breadth of light cell and diameter of the nucleus was found to be 11.91 μ, 10.12 μ and 4.09 μ.

Each follicle contained a viscous homogenous fluid the colloid. In the active follicle it was thiner and less viscous while in inactive it was thick and more in quantity. They often contained desquamated follicular cells. Vacuoles and irregular spaces were seen inbetween the colloid and epithelium.

In the vicinity of one of the thyroid gland, one small accessory thyroid was found. The microscopic structure of the accessory thyroid observed under present investigation were similar to those of thyroid gland proper.

Thyroid gland was richly supplied with blood vessel and nerve. Solitary ganglionic cells were observed in the stroma.

Follicular cells were P.A.S. positive, they were also positive for acidmucopolysaccharides, glycogen, protein, tyrosine, protein bound NH_2 whereas negative for lipid, calcium and iron. The light cells were positive for protein and were P.A.S. negative. They were also negative for protein bound NH_2 and glycogen. The colloid was P.A.S. positive and were also positive for protein, tyrosine, reactive protein bound NH_2, glycogen and acidmucopolysaccharides whereas negative for lipid, calcium and iron.

Dedicated to my Professor

Dr. R.C.P. Yadava, G.B.V.C.(Pat.),M.S.,Ph.D. (Michigan, U.S.A.), DIRECTOR, Livestock Research Station, Rajendra Agricultural University, Bihar, Principal and Head of the Post-graduate Department of Anatomy, Bihar Veterinary College, Patna whose unfathomable knowledge and deep devotion to teaching,research in field of veterinary anatomy have been a continuous source of inspiration.

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Kripa Shankar Roy

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Minor subjects :-	(i) Surgery (including Radiology)
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INTRODUCTION

The Indian buffalo was tipped for present investigation because of paramount importance of this species in Indian economy.

Holdings are small, agricultural practices are old fashioned and marketing of produce continues to be done with bullock carts in India. Cattle and buffalo are, therefore, indispensable for rural economy. They provide the required motive power for various agricultural operations including irrigation and rural transport. Viewing the value of milk, meat and hide production in India one observes that buffalo plays a great role in its economy. As such it is impossible to think of improving agriculture in India without having good cattle and buffaloes.

Randhawa (1958) described more than 50 percent (Rs. 1,135 crores) of our total agricultural income is derived from cattle. The total milk production by she-buffaloes is 31,71,26,647 maunds per annum as against only 22,87,43,075 maunds by that of cows. The value of buffalo meat production is Rs. 465 lacs and that of its hide is Rs.559 lacs per annum (Singh and Parnerkar, 1966).

Now-a-days Indian buffaloes are utilised in research laboratories in most of the veterinary colleges and research Institutions in India, after viewing their great role in our economy.

Maqsood (1952) pointed out that thyroid hormone influences reproduction and fertility not only by helping to maintain the pituitary hypophyseal gondal relationship but indirectly by affecting metabolic pool of nitrogen and energy.

Hafez (1968) indicated the correction of summer infertility in ram with thyroxine. In mammals fertility level and parturition have been seen to be sub-normal after thyroidectomy.

The most striking effects of thyroid secretion is its control of the metabolic rate of the body above a minimal value. It is not surprising that its influences carbohydrate metabolism and probably fat and protein metabolism. It is important also in growth of animal as whole especially through its effects on ossification center's and on the development of certain organs particularly the genital organs and the thymus. It also influences the functioning of the nervous system. There is certain inter-relationship with anterior pituitary. In some species the thyroid hormones is involve directly in pregnancy. In absence of the harmone the foetuses are reabsorbed or aborted. Reduction of the size, number and viability of young one gives evidence to an essential role of thyroid in pregnancy.

Viewing the importance this Endocrine gland it is imperative to study its micro-scopic structure.

The present investigation on histology and histochemistry of thyroid gland has been carried out under light microscope. As such ultramicroscopic structures are beyond the scope of the present work. Moreover, the structures of the golgi complex, certain enzymatic activity and radioautograph need further special histological and cytological studies.

Inspite of the above limitations the present investigation will certainly contribute towards up-to-date study of the anatomy of thyroid gland of the Indian buffalo with special reference to histology and histochemistry.

The author hopes that the paper will serve useful purpose to the research workers not only in the field of histology and histochemistry but also in the applied sciences.

REVIEW OF LITERATURE

Gross Anatomy

Vaughan (1907) described that at the first tracheal rings there was brownish red thyroid gland. It consisted of two lobes and was ovoid in shape. The minute vesicles were surrounded by plexuses of capillaries and connected by areolar tissue. The gland was very large in foetal life but its use was unknown.

The thyroid gland of dog was discussed by Bradley (1927). He stated that it consisted of two lobes – the right and the left. The glands were connected with the Isthmus ventrally on the trachea. The Isthmus was found to be frequently absent but when it was present, it connected the more caudal part of the two lateral lobes.

Jones (1946) described that in man the Isthmus was constant as regard size and position. According to him the depth of gland was ¼" to 1" and breadth was about ½". It connected the lower parts of the anterior border's of the lateral lobes but did not reach the base.

Johnston & Willis (1946) described that the thyroid gland of man was highly vascular organ situated at the front and sides of the lower parts of neck opposite 5th, 6th, 7th cervical and 1st thoracic vertebrae. It was attached by the trachea with pretracheal and deep cervical fascia. It was having two lobes – the left and the right, connected by Isthmus. Its average weight was 30 gm. Each lobe was about 5 cm. long, 3 cm. in width and 2 cm. in thickness.

Neal and Rand (1947) described the shape of the thyroid gland as to be U or H shaped, depending on the situation of the Isthmus in relation to the trachea in man. Its average weight was found to be 34 gms.

According to Sisson and Grossman (1961) the thyroid gland of ox was softer in texture than horse and was pale in adult and dark red in calf. Two glands joined with Isthmus. Lateral lobes were irregularly triangular and flattened than in horse. The lateral lobes were about 3 inches or more (ca 8 cms.) in length and half an ounce (ca 14 to 15 gms.) in weight. The Isthmus was constantly present and glandular. In fowl the two lobes of the thyroid gland was at the root of the neck and was oval in shape.

Turner (1961) described that the thyroid gland in low vertebrate were not incapsulated and organised into compact mass. The thyroid follicles of lamprey's and boney fishes tend to be dispersed along the ventral aorta. The thyroid tissue of certain Teleosts was extremely mobile and may dispersed to eye, brain, spleen and kidney.

Fujita and Kamiya (1963) reported that the thyroid gland of old female elephant was horse shoe shaped and was clearly sub-divided into distinct lobules.

Last (1963) described the presence of pyramidal lobe in thyroid gland of man. He stated that the small portion of the glandular substance often projected upward from the Isthmus, generally left to the midline. It was named pyramidal lobe.

Raghawan (1964) described that in thyroid gland of ox was vascular ductless gland situated in contact with larynx. It was reddish brown in adult and red in calf. It was having right and left lobes connected by Isthmus. Each lobes were about 3.7 to 5 cm. long, 2.3 to 3.7 cm. wide and 3 to 6 cm. thick. Isthmus was in form of transverse glandular band. It was about 6 to 12 mm. in width and was situated across the inferior face of the trachea. It had two borders – the dorsal and ventral, two surfaces – the lateral and medial and two extermities.

Karski (1964) examined thyroid gland of monkey. In 80% cases the two lateral lobes united in their lower or medial portion by an Isthmus and in remaining 14.7% separate lateral lobes were present. The lateral lobes were situated between the lower one third of the posterior margin of the thyroid cartilage and 4th tracheal cartilage.

Pantic and Stosic (1966) noted the average weight of thyroid gland of Roe-Bucks to be 1.1 gm.

Microscopic Anatomy

Capsule

Maximow and Bloom (1952) described that the external connective tissue capsule of the thyroid gland continues into the surrounding cervical fascia. It was connected by loose C.T. with another layer of dense connective tissue adhere intimately to the gland.

Trautmann and Fiebiger (1957) noted that thyroid gland of domesticated animals were surrounded by connective tissue capsule, which sends septa of varying thickness to the interior of gland.

Das *et al.* (1965) investigated thyroid gland of Bull and Bullock. According to them thyroid gland was enclosed in a thick capsule made up of collagen and reticular fibers with a few smooth muscle.

Arey (1968) described that thyroid gland of man was covered by Fibro elastic capsule which sends delicate septa inward. Areolar and reticular tissue provided a thin highly vascular stroma.

Ham (1969) described that thyroid gland of man was covered by two capsule. The outer one was continued as pretracheal fascia and which inturned was part of deep cervical fascia. The inner capsule was regarded as the true capsule of the gland. It was made up of fibro elastic connective tissue. Capsule sends septa into the gland provided enter support and was carried blood vessel, Lymphatic and nerve into substance of the gland.

Follicle

Norris (1916-18) investigated the morphogensis of the thyroid follicle. He pointed out that the first secondary follicle appeared in foetuses of 56 mm. in length in man. Further growth of the gland took place largely by increased in size of individual follicle rather by increased in their number.

Bhatnagar et al. (1955) reported that in autum the diameter of the thyroid lobules were significantly lower than in other season in buffalo. In summer diameter of the thyroid lobule was significantly more than in winter and spring.

Barbara and Barbara (1957) noted the mean diameter of thyroid follicle in sparrow were 20.4 /u and 35.9 /u. Large follicles were tend to be common in thyroid with low activity.

Trautmann and Fiebiger (1957) described that in young animals the follicles were smaller than in adult. Between the follicle there was fat free connective tissue containing blood and lymph capillaries and primitive cells. The follicle were completely closed by net work of reticular fibers. They were usually spherical or ovoid in shape.

Tomonari (1959) found that in mammalian thyroid the peripheral follicles were large and central ones were smaller and immature.

Yust (1960) described that thyroid lobes and Isthmus were made up of follicles which were rounded in shape and varying from .05 mm. to .5 mm. in diameter.

Harrison et al. (1962) noted that mean diameter of the follicle increased from about 0.050 mm. to late foetal stage to

0.130 mm. in adult seal.

Copenhaver (1964) described that follicles were structural units of thyroid gland. The follicles were vary in shape and size but they were usually irregularly spheroidal. Stroma consisted of connective tissue, blood vessel and lymph capillaries. Follicle were lined by simple epithelium and filled with gel like material "Colloid".

Das et al. (1965) reported that in bull lobules were made up of numerous follicles and enter follicular primitive cells. The shape of follicles were mostly circular and oval. The smaller follicles were mostly located towards the periphery and larger one in the centre. Connective tissue fibers between the follicles were scanty. In bullock the enterfollicular primitive cells were more abundant. The follicles were arranged in an irregular manner and were smaller in comparison to those in the bull.

Mosier and Richter (1967) found that smaller follicles were located in centre of the thyroid gland in wild and domesticated norway rats.

Srivastava and Sathyanesan (1967) found that thyroid follicle of varying size were scattered throughout the kidnies in the Indian Mudeel Amphiprious Cuchia (H.A.M.).

Isler et al. (1968) while noting the architecture of thyroid gland cited that Jones (1836) found thyroid follicles were made up of one layer of cell surrounded a mass the colloid. Thus opposing Lalouettes (1950) concept of follicle as branching sac a controversy developed which was not dispelled by subsequent reconstruction studies of various order (Streiff 1897; Norris 1916, 1918; Wilson 1927; Rienhoff 1929; Moritz 1931; Hammer and Loescheke 1934; Ewe 1936; Heidenhains(1921) observe that the follicle could exist side by side without being separated by connective tissue was confirmed by Yagizawa (1956).

He further pointed out that in some places the base of epithelium were in direct contact with base of epithelium of an another follicle and that was due to absence of C.T. and basement.

Bloom and Fawcett (1968) described that thyroid gland was composed of spherical cyst like follicle 0.02 to 0.9 mm. in diameter. In animals other than man the follicles were more uniform in size. The follicles were seen to be enclosed by delicate network of reticular fibers.

Ham (1969) described that follicles were surrounded by a basement membrane which is P.A.S. positive.

Harrison and Young (1970) investigated that the thyroid gland of Dolphin were made up of small irregular follicle with a diameter 35 to 125 /um. The follicles in some areas of the gland were large with marked folding of the epithelium. Large follicles upto 350 /um. in diameter were seen in one Dolphin. Occasional follicles were filled with cellular debris. The enterfollicular spaces had rich capillary net work, collagen fibers and fibroblast.

FOLLICULAR CELL

Chauveau (1891) described that in domesticated animals parenchyma were divided into lobules. In foetuses it was lined by polygonal cells with a large nucleus and were containing a granular fluid.

Nonidez (1932) investigated an additional cell of the thyroid gland in greater detail. They were commonly called "Parafollicular cells".

Dempsey (1954) described two kinds of follicular cells in man. They were :

(i) The chief cells may be columnar, cuboidal or flattend depend upon the degree of stimulation of the gland.

(ii) Colloid cells of langendorff were present in all animals (Amphibia, reptiles, birds, mammals). They were laterally compressed narrow cell with small pycnotic nucleus and a dense acidophilic cytoplasm. They were smaller in number and regarded as degenerating cells.

Bhatnagar <u>et al.</u> (1955) noted height of the thyroid follicular cell in buffalo in all the four seasons. The average height were 3.69 μ in summer, 3.09 μ in autum, 4.47 μ in winter and 4.79 μ in spring. In autum the height of the epitheliul cell was significantly lower than in any other season.

Trautmann and Fiebiger (1957) described that cell lining of the thyroid follicle had large round nucleus. They were closely opposed to inter follicular connective tissue or capillaries. The cells were cuboidal or low columnar adjoined by terminal bars. A marked proleferation and desquamation of follicular epithelium were observed in Bovine thyroid.

Welch et al. (1958) found that rat treated with progesterone had higher epithelial height than normal rat, varying from 8.1 /u to 8.6 /u (normal 7.6 /u).

Tomonari (1959) investigated histological structure of thyroid gland in cow, pig, horse, cat, dog, rabbit, rat, and guinea-pig. The large follicle had flattend epithelium and were not active in colloid formation and reabsorption while small follicle had columnar epithelium, which were presumably synthesizing and secreting certain substance. The discharge of intra cellular thyroglobulin was carried out by secretory mechanism identical with that in protein cells of Exocrime glands.

The apocrine secretion frequently observed in the cow, horse and rat. The intra follicular vacuoles seem to develop not from apocrine secretion but also from accumulation of tiny vacuoles eliminated directly through the cell membrane. The enter cellular discharging canaliculi seen in human thyroid were not found in these animal except rat.

Kanaya (1960) observed that follicular cells of the 5 months old human foetuses thyroid gland were cuboidal or columnar. Latter on these cells become small and low cuboidal. Secretion thought to be apocrine type although in some nearly halocrine type of secretion was observed.

Mukerjee et al. (1960) found that there was seasonal changes in the histology of thyroid in goat and ram. The thyroid activity was decreased in summer and autum but increased in winter and spring.

Kamar (1961) investigated thyroid activity of growing male thickenes with the advancement of age. There was an increase in cell size, number and thickness of epithelium. Although there was decreasing trend with age advancement from 24 weeks age onward.

Harrison <u>et al.</u> (1962) noted that the cow thyroid epithelial cells were uniformly columnar and higher than any group of common seal. The outline of individual cell was often indistinct and in section the epithelium thus appeared more than one cell deep. Nuclei were small and intensely basophilic and majority have a clear nucleoplasm. The apical cytoplasm containing numerous pale brown granules or droplets. All glands contained varying degree of connective tissue and amorphous hyaline materials between the follicle. Capillary blood supply was well developed.

Copenhaver (1964) described that cellular height of follicles depending upon activity of the thyroid gland. In normal gland they were cuboidal. But when a follicle was distended with colloid they became low cuboidal or even squamous. In highly active glands the cells were tall columnar. The nuclei and cell membrane were distinct and terminal bars were present. In addition to follicular cell another type of cell were present called colloid cell of langendorff. These cells were filled with colloid having pycnotic nuclei the degenerating cells.

Raghawan (1964) noted that thyroid follicle of young animal (ox) was lined by polygonal cells with a large nucleus. In adult animals (ox) the vesicles, were distorted and epithelium were less evident but they were uniform.

follicular lumen. Basement membrane were found surrounded the follicular cells.

Youson and Van Heyningen (1968) noted that follicular cells of rat thyroid gland were low to high cuboidal or pyramidal in shape with truncated apex. Their round and sometimes irregular nucleus was basely located the nucleus contained a prominent nucleolus.

Bloom and Fawcett (1968) described that the epithelial cells of human thyroid varied in height but were commonly low cuboidal to squamous. In general the epithelium tended to be squamous when the gland was underactive and columnar when it was overactive. The nucleus of the gland was spheroidal, centrally situated, poor in chromatin and contained one or more nucleoli. The cytoplasm was basophilic.

Ham (1969) described two different kinds of cells present in epithelial walls of the follicles.

(i) The great majority approximately 98% in rat were normally cuboidal epithelium and were consumed in the production and absorption of the colloid materials.

(ii) The second kind of cells were known as light, parafollicular or C cells. They were larger and had paler cytoplasm than low cuboidal epithelial cells.

Harrison and Young (1970) found marked folding of the epithelium in the follicle of thyroid gland of Dolphin. Occasionally the follicles were filled with cellular debris. In addition to follicular cell a second type of cell was recognised as light or parafollicular cells.

Calvert and Isler (1970) investigated that the thyroid parenchyma of rat was made up of mostly thyroxine producing follicular cell and calcitonin producing light cells.

LIGHT CELLS

Nonidez (1932) investigated the origin of parafollicular cell a second epithelial component of the thyroid gland in dog. The silver nitrate method of cajal had disclosed the presence of large epithelial cell with argyrophilic granules in puppies while working on innervation of the thyroid gland. These cells originated in the follicular epithelium and later on migrated into follicular space remaining close to the follicle hence they called 'Parafollicular cells'.

Nonidez was not able to detect mitotic figure in the parafollicular cells. Their number increased through differentiation of new cells in the follicular epithelium. The argyrophilic granules of the parafollicular cells had greater affinity for silver nitrate than follicular cells.

Owing to their great size the parafollicular cell could be readily identified in sections of the thyroid stained with haematoxylin and eosin. After leaving the follicular wall the parafollicular cell did not seem to possess definite polarity. Some cells were roundish other may show polygonal in outline.

In puppies aside from the differentiating parafollicular cells the other element in the follicular wall were quite uniform in aspect. The distinction between chief cell and colloid cell first reported by langendorff.

The nature of the argyrophilic granules was discussed and the conclusion was reached that they probably represented the antecedent of an endocrine secretion poured directly into the vesicle.

Stux et al. (1961) observed that shortly after an injection of thyrotrophic hormone all the follicular cells of the thyroid gland of rat contained numerous intracytoplasmic colloid droplets whereas a few cells referred to as "light cell" did not contain any.

With most of staining techniques the light cell showed a large pale nucleus and faintly stained cytoplasm. They were located within follicle but were not in contact with the colloid. They lies singly or in small groups between the follicular cells and the basement membrane or among the follicular cells but even in this case they were separated from the colloid by a layer of follicular cells cytoplasm. Since light cells were within the follicle they were considered to be epithelial in nature and therefore were neither ganglionic cells, parathyroid cells nor embryonic cells.

The light cells instead of well defined cuboidal or columnar arrangement they were not polarized and their shape varied. They often had a smooth circular or oval outline but occasionally were polygonal or spindle shaped. Their nuclei were round and usually larger than epithelial cells and often contained an unstained vacuoles. Mitosis of these cells were seen but seldom. Their cytoplasm was lightly stained. It did not contain colloid droplet and appeared homogenous although a small basophilic mass was occasionally seen.

Gabe (1961) demonstrated the presence of parafollicular cell in thyroid gland of birds, reptiles and amphibia.

Sarkar and Isler (1963) noted that the light cells of thyroid gland of the rat increased in number after treatment with somatotropic hormone and after hypophyscetomy. The presence of few mitotic figure in the light cells indicated that some of light cell originated from light cells. It was found that their rate of proleferation was much too low to account for the over all growth of the light cell population. Most newly appearing light cells present in the thyroid gland presumably originated from the follicular cells.

Young and Leblond (1963), Tashiro (1964), Stoekel et al. (1967), Shively and Epeling (1969) described the ultra structures of parafollicular cells in mammals.

Amat and Vazquez (1965) noted that light cells were present with large nuclei and very feeble stained cytoplasm in the thyroid gland of guineapig. These light cells were not in contact with the colloid. The presence of light cells in the follicular wall was interpreted as a sign of thyroid hyperactivity.

Amat and Garcia-Lacrew (1966) found that all the cells accumulation in interfollicular and parafollicular spaces were not result of trangential section of follicle in pig. Some of these accumulation were scooped by a diverticulum which protruded from an adjoining follicle. Which was refilled with inter stitial tissue. They were identified as 'Nonidez' parafollicular cells.

Pearse (1966) noted that a population of cells exists in the thyroid gland of dog and other mammals which were entirely distinct from the population of follicular or acinar cells. They were characterised cytochemically by a content of basic protein and high α glycerophosphate dehydrogenase (α G.P.D.). These cells the 'C cells' respond to high blood calcium level by increased secretion. It was postulated that this product was thyrocalcitonin the calcium regulating hormone of the thyroid gland. Three distinct type of parafollicular cells were described by Pearse. (A') with high acid phosphatase content and trace of α glycerophosphate dehydrogenase and mitochondria. A second type of (C) had most of acid phosphatase. The third (C') had much low α G.P.D. and no appreciable acid phosphatase.

Aoi (1966) found that in primates the parafollicular cells were generally ellipsoidal in shape and larger than ordinary follicular cells. They were in direct contact with the follicular cells but did not line the follicular cavity. Secretory granules were distributed in abundance evenly throughout the cell bodies.

Pantic and Stosic (1966) investigated that parafollicular cells were very frequent in Deer and buck thyroid gland. The presence of big nucleolus in nuclei of these cells and a basophilic cytoplasm pointed out an active role of these cells.

Halmi (1966) described that there were two kinds of epithelial cells in the thyroid follicle - the follicular cells proper and cell which had many names but were best described as light cells. They were never in contact with colloid and lack of polarity believed to be derived from follicular cells.

Bussolati et al. (1967) noted that calcitonin was present in the cell of pig and dog thyroid.

Carvalheira and Pearse (1967) noted that pig thyroid contained a second epithelial cell in addition to the follicular (acinar) cells. This was referred as 'C cell'. Cytochemically it characterised by high phospholipids contents unlike the 'C cell' of rodent and dog thyroid it possess no cholenes terase. The 'C cell'of pig thyroid was intrafollicular, epifollicular and as well as parafollicular.

Calvalheira and Pearse (1967) gave comparative cytochemistry of 'C cell' of mammalian thyroid parathyroid complex. In dog, rat, rabbit and guineapig the 'C cell' contained a strong chelenesterase.

Matsuzawa and Kurosumi (1967) suggested that the paraffolicular cells of rat were the most intimately concern with function of the thyroid gland. Which lower the calcium concentration in serum in response to the acute administration of calcium into the blood stream.

Saljelid (1967) found demosome were present inbetween adjacent follicle cells as well as between parafollicular cell, but were not observed along the contract between a parafollicular cells and follicular cells.

Beskid and Roseizewska (1968) noted the morphological and histochemical properties of 'C cell' of man, pig, ox and dog. C cell were characterised by strong activity of glycerophosphate dehydorgenase and phospholipids.

Beskid <u>et al</u>. (1968) found that the C cell in normal thyroid of man occurred frequently among the cell lining of the vesicles in the para or intervesicular spaces.

Bloom and Fawcett (1968) described that in addition to principal cells other types of cell present in thyroid fallicles were first noted by Baber (1877) and Hurthle (1894) they were commonly called parafollicular cells, motochondria rich cells or C cell. They were rich in mitochondria and α glycerophosphate dehydrogenase probably secrets thyrocalcitonin.

Kameda (1968) investigated the parafollicular cell of dog, cat, rabbit, rat, mouse and guineapig by Davenport silver impregnation method.

Kracht <u>et al.</u> (1968) found that in mammals the thyrocalcitonin was synthesized by C cell which constituted a second epithelial cell system of the thyroid gland derived from ultimobrachial body.

Young <u>et al.</u> (1968) confirmed the presence of light cell in addition to more common follicular cells in pig thyroid. In pig, out of 14,520 follicular cells 142 light cells were seen. They occur either singly or in a group of two or three. They were elongated and were situated towards the outside of the follicle but within basement membrane. They were usually elongated in length with dense nuclei. Colloid droplets were never seen in the cell cytoplasm.

Gabe and Martoja (1969) noted that calcitonin cells were wide spread throughout most of the laterals lobes of the thyroid gland of garden dormouse. They were larger than follicular cells and their cytoplasm were more or less filled with cynophilic secretory product. They exhibited strong argyrophilia and stained metachromatically with Toludine blue.

Steven et al. (1970) demonstrated the presence of follicular and canalicular spaces within a group of parafollicular cell. The parafollicular cells (C cells) of the dog thyroid were believed to be producer of thyrocalcitonin.

COLLOID

Dempsey (1954) described that colloid filling the lumen of the follicle was a hyaline material which stained intensely with eosin and haematoxylin. By Azan, the colloid was stained by one of the acid dyes but its colour varied with state of activity. The thick colloid (inactive) stained brilliantly with Azocarmine. While the thinner and less viscous colloid (active) took bluish tint with aniline blue. With basic dyes inactive colloid exhibited basophilia and active colloid also basophilia but weakly. So colloid was supposed to be amphoteric.

Trautmann and Fiebiger (1957) described that follicle contained colloid a viscous fluid rich in Iodine secreted by the cells. Desquamated epithelial cells occurred in the colloid. Proleferation and disquamation of follicular epithelium was observed in bovine thyroid. The vacuoles of colloid were fixation artifacts. Colloid were usually homogenous acidophilic but sometimes showed varying degree of basophilia.

Von Heyningen (1961) investigated that colloid appeared between 15 to 16 days of age in mouse. Whereas production of thyroxine and follicle appeared between 16 to 17 days of age.

Copenhaver (1964) described that colloid in different glands or even in the follicle of a single gland may show tinctorial difference. In activated glands the colloid was predominantly basophilic but in inactive glands the colloid was acidophilic. Desquamated or phagocytic cells were present in the colloid.

Das et al. (1965) noted that in bull each follicle was filled with a homogenously staining colloid materials which was acidophilic. But in bullock colloid stained lightly and presented large number of vacuoles. In many follicle colloid shrunkened detached from the follicular wall.

Bloom and Fawcett (1968) described that the fresh colloid was aplically homogenous. Occasionally it contained desquamated cells and rarely macrophages. Multiple staining had observed due to local difference in concentration of protein.

ACCESSORY THYROID GLAND

Trautmann and Fiebiger (1957) described the presence of accessory thyroid gland in domesticated animals. They had similar structure and function as the regular thyroid gland and were specially common in poultry.

Karski (1964) noted the presence of accessory thyroid gland in monkey. 4.4% accessory thyroid glands were found in the neighbourhood of the lateral lobes of the thyroid glands in monkey.

Sanson (1967) found accessory thyroid gland was present at the base of the neck in between the jaws in 4 out of 17 normal adult goats. Thyroid-parathyroidectomy were performed on 16 immature animals out of which 13 were presented residual or accessory thyroid tissue 12 to 14 months latter.

BLOOD SUPPLY

Trautmann and Fiebiger (1957) described that the blood vessels were numerous and capillaries forms dense network, which surrounded the follicle in basket like fashion. The Lymph vessels originated as perifollicular capillaries net. They continued in the supporting frame work and communicated with Lymphatic network in the capsule.

Ivanova (1962) noted that the arterial supply of human thyroid gland divided into two zones :-(i) the outer zone where the main arterial stem and major branches occurred on the upper and lateral surfaces of the gland. (ii) the central zone where the arterial vessels were of small diameter.

Nazarova (1962) found that interlobular connective tissue contained arterio-venous anastomosis. Each follicle had its own independent capillary network. The efferent venules of the lobule was situated opposite the afferent arteriole.

Cecio and Caputo (1963) noted that the thyroid blocking structures were detected in blood vessel, formed by endothelial cells, valves, a-v anastomosis and myo-epithelial cells in human, bovine, and ovine thyroid gland. The venous valves were located in the superficial veins of the gland.

Baranova (1964) noted a very complex arrangement of the perifollicular venous network in toxic goiter. The vessels of the capillary network of the follicles were numerous.

Faller (1964) discussed the angioarchitecture of the thyroid gland in new born infants. The lobules were supplied by single lobular artery. Distially the lobular artery divided into numerous intra lobular branches from which short follicular arteries feeding the capillaries network.

Prokopchuk (1966) noted that human thyroid were penitrated by inter lobar arteries which originated from the capsular vascular network. From Interlobular arteries of 4th, 5th order smaller branches sprang to supply lobules of the thyroid gland; each lobule being supplied by 1-4 arteries with the diameter of 20 to 50 μ. The intralobular arteries divided dichotomically into interfollicular arteries with diameter of 18-25 μ. Each of which divided into 4 to 10 perifollicular basket like plexuses.

Wenzel (1968) noted that in rabbit capillaries enclosed small groups of two to five follicles. The connective tissue surrounding these follicles were rich in Lymph capillaries. Lymphatic with valves were found in the trabecular (interlobular and interlobar) connective tissue in the external capsule.

NERVE SUPPLY

Trautmann and Fiebiger (1957) described that nerve which often bear ganglion supplied fibers to the blood vessel and to follicular epithelium. But their influence on secretion was unproved.

Okamato (1959) noted the presence of myelinated fibers immediately adjacent to the follicles in thyroid gland of man and adult dog. These nerve fibers showed in the interstial tissue specific and simple or arborized termination.

Kladienko (1962) observed that the thyroid gland of cattle possessed its own intrinsic nerve apparatus which consisted of 3 types of nerve ganglia.

Agarkov and Nosov (1965) found that the nervous apparatus of the thyroid gland of man consisted of numerous nerve bundles and fine nerve fibers. Nerve pleuxses freely distributed in the parenchyma.

Sarrat (1965) noted that the ganglion cell was present in thyroid gland of rat between follicle. They appeared both in solitary or forming groups with 3-4 cells. Surrounding them a thin connective tissue sheath was visible.

Diculesco _et al._ (1966) noted that the thyroid gland of the horse, ox and dog was richly innervated by cholinergic and adrenergic fibers. In the capsule both type of fibers appeared in the form of nerve trunk and nerve bundles. They penitrated through the hilus and branched out in form of rich vascular plexuses. They penitrated as fine filaments into inter follicular septa.

Makita _et al._ (1966) found many small nerve bundles in the connective tissue of the capsule. Among them sensory nerve fibers were seen. Small number of nerve cells were seen in association with nerve bundle entering the gland. The terminal branches ended with club shaped nerve endings. Fine perifollicular fibers as well as thicker afferent fibers were seen around the follicles.

HISTOCHEMISTRY

P.A.S. Positive materials

Bollet et al. (1959) noted the presence of acid mucopolysaccharides in dog thyroid gland.

Gabe (1959) found that parafollicular cell of canine thyroid contained cynophilic secretory granules, which can be stained by mallory Heidenhains azan. The cytoplasm contained aromatic amino acids, protein bound sulph-hydryles and P.A.S. positive glycoprotein. No sudanophilic lipid were detected.

Idelman (1963) investigated macrothyrocyte in Bos Ovis and sus. They were rarely localised parafollicularly. Histochemically in the section of these macrothrocytes a carbohydrate component different from glycogen and acid mucopolysaccharides was demonstrated. Carbohydrate and protein were probably associated with glycoprotein which was precursor of thyroglobulin.

Belanger and Bois (1960) noted two fraction of colloid in guineapig thyroid both were P.A.S. positive.

Belanger and Bois (1964) gave comparative histochemistry of rat thyroid fixed in different fixatives. They revealed a proportion of protein, carbohydrate and iodinated materials.

Van Heyningen (1965) found two types of globules in follicular cell stained by P.A.Silver methenamine and P.A.schiff method. Their periodic acid reactivity is due to some glycoprotein as that of follicular colloid. The second type comprises of fairly electron opaque globules.

Mosier and Richter (1967) noted that intracellular and intrafollicular colloid were P.A.S. positive in wild and domesticated Norway rat.

Seljelid (1967) found that when a section of normal gland of rat stained by P.A.S., the follicular colloid gave a strong positive reaction. In hypophyscetomized gland, small P.A.S. granules were found in follicular cells.

Tavares and Carvalheira (1969) investigated that metachromatic staining with Toluidine blue and result of colloidal iron method showed that acid polysaccharides were consistently found in these cells.

PROTEIN

Robbins *et al.* (1959) noted the presence of two types of iodoprotein other than thyroglobulin in normal human and sheep thyroid tissue.

Feyrter and Zischka (1964) found the presence of lipids and lipoprotein in human thyroid.

Belanger and Bois (1964) noted that colloid of rat thyroid revealed the presence of basic protein when stained by toluidine blue and also by bromphenol blue without mercury.

Nadler and Young (1964) traced newly synthesized protein by radioautography in thyroid gland of rat.

Lietz and Zippel (1969) demonstrated that C cells in the mammalian thyroid contained acid cytoplasmicproteins which can be cytochemically demonstrated by metachromatic staining reaction with toludine blue.

IODINE

Lampe (1961) found that the onset of Iodine uptake in rabbit foetuses fall on the 17th and 18th day and in rat on 18th day.

Iodine incorporation began with the development of follicle and increased untill birth.

Desmarais and Laham (1962) developed a new staining technique for the thyroid gland involving the use of two components of mallory C.T. Stain, Aniline blue and orange G in reversed proportion various indices such as incorporation of I^{131}, Epithelial cell height and number of blue and yellow staining follicles and total number of follicles were used to test the validity of the colour reaction in the colloid. The colloid materials which stains with aniline blue corresponds to iodinated thyroglobulin while the Yellow staining materials appeared to be devoid of biologically active iodinated amino acid.

Rabision and Davis (1969) determined the concentration and distribution of iodine in various sizes of follicles in rat thyroid. The concentration of Iodine ranged from .9 to 2.1% by weight and distributed uniformally across the colloid.

FAT

Ando (1960) found that fat droplets were present in the supra nuclear position of the follicular cell in rat thyroid.

Pearse (1966) noted the presence of high level of phopholipids in C cell of thyroid gland.

CALCIUM

Culling (1963) described that calcium may be found in fibrosis and Tuberculosis foci and thyroid gland.

ZINC

Krasovskii (1967) determined zinc level in thyroid gland histochemically. In the small follicule which stained more intensively when zinc was detected.

MATERIALS AND METHODS

Source of Animals:

For the present investigation thyroid glands from twenty Indian buffalo of different ages were collected. Out of twenty, ten were male buffalo calves between 2 to 3 years of age and rest ten were adult female buffaloes between 9 to 14 years of age. All the animals chosen for present study were healthy and apparently free from disease.

The thyroid glands of adult female were collected from slaughter houses and thyroid glands of male calves buffaloes were collected from surgery and Anatomy Department of B.V.College, Patna. The glands were collected as soon as the animals were slaughtered or killed to prevent decomposition and were put in various fixatives.

Fixatives : The following fixatives were used during the course studies :

(i) Formalin :-

(a) 10% formalin aquous

(b) 10% Neutral aqueous formalin

(c) 10% aqueous buffer formalin

(ii) Bouin's Fluid.

(iii) Heidenhain's 'Susa'.

(iv) Helly's Fluid.

Technique :

Gross :- Both left and right thyroid glands were weighed with the help of physical balance. The maximum length, width and thickness of each right and left glands were taken by slideclipper. The average and standard error of both sexes were calculated.

Histological

Preparation of Blocks :- After the required time of fixation in the various fixatives two pieces were taken from each of the left, right and Isthmus of thyroid glands.

Water and alcoholic wash :- Some blocks thus obtained were then washed in running tap water for 6 to 12 hours and then put in jar containing 70% alcohol. Some blocks were kept directly into 70% alcohol without washing in running water.

Dehydration and clearing :- Tissue of thyroid gland after getting rid of various fixatives by above method passed through different grades of Ethyle alcohol as follows for dehydration.

(1) Jar I containing 70% alcohol - 12 hrs.

(2) Jar II containing 80% alcohol - 12 hrs.

(3) Jar III containing 95% alcohol- 12 hrs.

(4) Jar IV containing absolute alcohol - 2 changes (4 hrs. each).

(5) Jar V - Cedar wood oil - 24 hrs.

(6) Jar VI Cedar wood oil - 24 hrs.

A few blocks were dehydrated and cleared by Acetone and Benzene schedule as follows :-

Jar I Acetone I - 15 minutes.

Jar II Acetone II - 15 minutes.

Jar III Acetone and Benzene (1:1) 15 minutes.

Jar IV Benzene I - 15 minutes.

Jar V Benzene II - 10 minutes.

Infiltration :- After the dehydration and clearing the tissue of thyroid gland were passed through three changes of paraffin at

$60^{o}C$ in paraffin oven as follows : -

Jar I containing $60^{o}C$ paraffin - 6 hrs.

Jar II containing $60^{o}C$ paraffin - 6 hrs.

Jar III containing $60^{o}C$ paraffin- 8 hrs.

Embedding :- Small paper boats were made and tissues were embedded in hard paraffin ($60^{o}C$ B.D.H.).

Sectioning :- Usually sections were cut at 5 to 6 μ for histological studies and thick section of 10 to 15 μ were cut for histochemical studies.

Some Frozen section of thyroid tissue were cut for histochemical (lipids) studies.

Staining :- The following stains were used for histological and histochemical studies :-

(A) Histological stains:

(I) Harris's haematoxylin and eosin used as routine method of staining.

(II) Gomori's aldehyde fuchsin for elastic fibers (culling 63).

(III) Azocarmine mallory stain (Gurr 62) for connective tissue and colloid.

(IV) Gridley's reticulum stain for reticular fibers (Gridley 1951).

(V) Weigert and van Gieson stain was employed for collagen fibers and muscle fibers (culling 63).

(VI) Thionin for nerve tissue and nerve tract (Gurr 62).

(VII) Mallory's stain for connective tissue (culling 63), (Preceded by Haematoxylin regressive staining).

(VIII) Metzner's method for mitochondria (Sumner and Sumner 1969).

(B) Histochemical stains :-

(a) Demonstration of polysaccharides

(i) P.A.S. reaction on section treated and untreated with human saliva.

(ii) Best's carmine (Pearse 1968) for glycogen.

(b) Acid mucopolysaccharides :-

(i) Alcian blue methods for acid mucopolysaccharides (Pearse 1968).

(c) Demonstration of Deoxyribonucleic acid (D.N.A.) Feulgen reaction (culling 63)

(d) Von Kossa's method for calcium deposits (Pearse 68)

(e) Perls' method for Ferric iron (Pearse 68)

(f) Protein :-

(i) Mercury bromphenol blue method for protein (Pearse 68)

(ii) Ninhydrin-Schiff method for protein bound NH_2 (Yasuma & Itichikawa 53; Pearse 68)

(iii) Millon Reaction for Tyrosine (Sumner and Sumner 1969)

(g) A stain developed by Desmaris & Laham (1962) for staining of the thyroidal colloid and its iodine content. The following was composition of the stain:

Phosphotungstic acid	1.0 gm.
Aniline blue	1.0 gm.
Orange G	.5 gm.
Distilled water	100 ml.

The pH of staining solution was constantly kept at 2.65.

(h) Lipid :-

(i) Alcoholic Sudan IV (culling 63)

(ii)Lillie and Ashburn's isopropanol oil red o method (culling 63)

(C) Stains used for light cells :-

(i) Weil-Davenport's technique (culling 63)

(ii) Cajal stain (Davenport 69)

(iii)Heidenhain's azan stain (culling 63).

Mounting :- The mounting of sections were made in D.P.X. (BDH) and glycerine Jelly.

Measurements :- Diameter of follicles and follicular cells were measured with help of an "ocular micrometer", which was standardised with a "stage micrometer". On the average six measurements were considered statistically significant so only six slides from each animals, 10 male calves and 10 adult female buffaloes were selected for the measurement of different structures. Thus average of six measurements from each animal of different structures gave the measurement of particular structure of that animal.

RESULTS AND DISCUSSION

Gross Anatomy

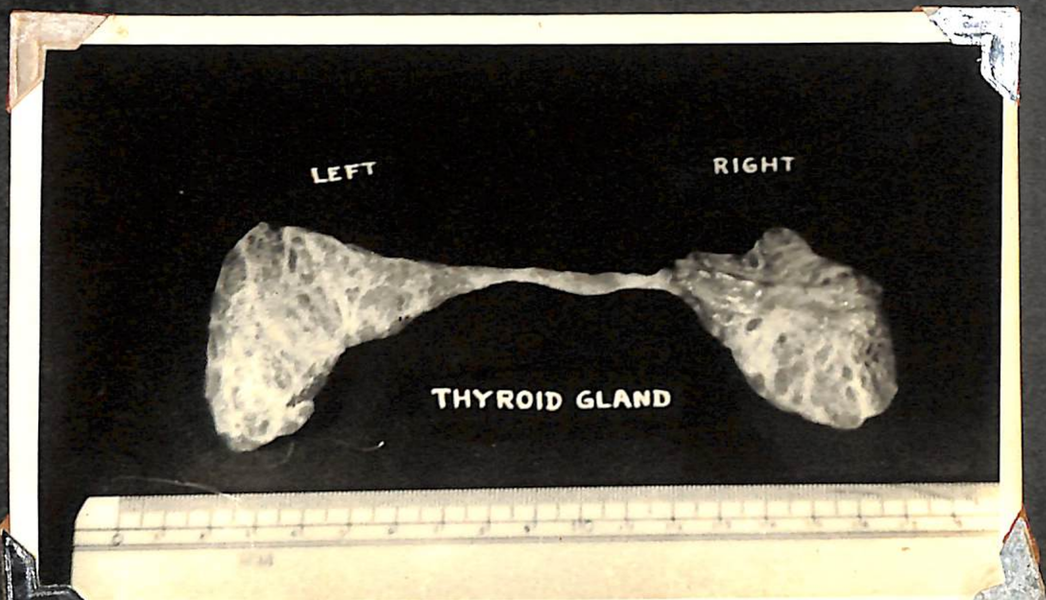
The thyroid gland of Indian buffalo was found to be a highly vascular endocrine gland consisting of two lobes, the left and right connected by an Isthmus. The lobes were situated in the lateral aspect of the trachea close to the trachio-laryngeal junction. The peritracheal and deep cervical fascia enchroached upon the lobes formed their capsule. The shape, size and position of thyroid gland varied greatly but they usually resembled like English letter U or H when removed and spread over a flat surface. The thyroid glands of young ones were dark red in colour and pale in that of adults. The surface of the gland was clearly sub-divided into distinct lobules. These lobulations were more marked in adult than in young (Plate II & III).

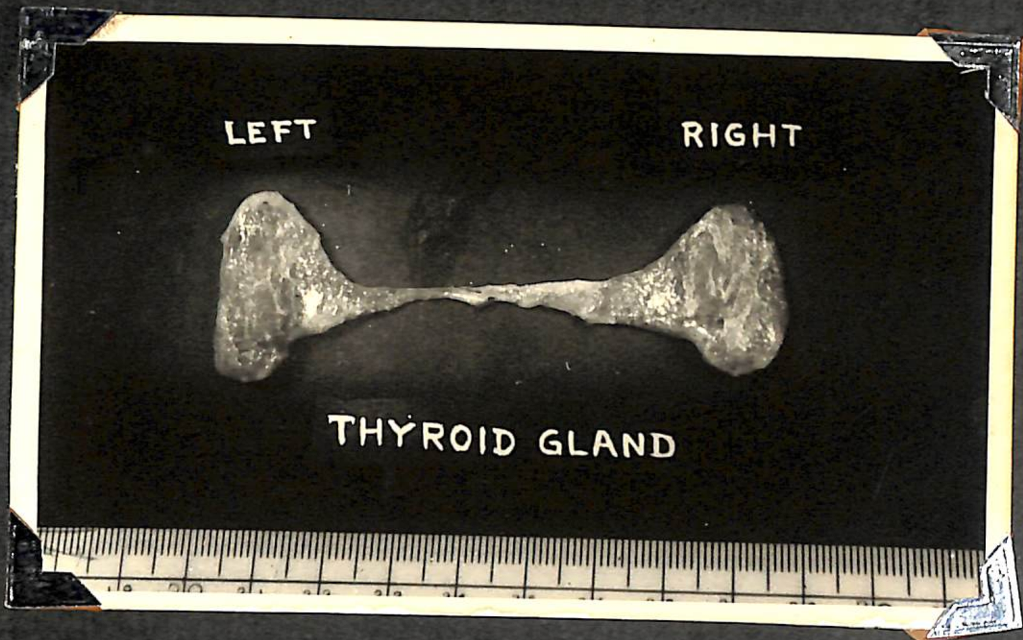
The lateral lobes were irregularly triangular in outline and flattened lateromedially. Each lobe had two surfaces, the lateral and medial.

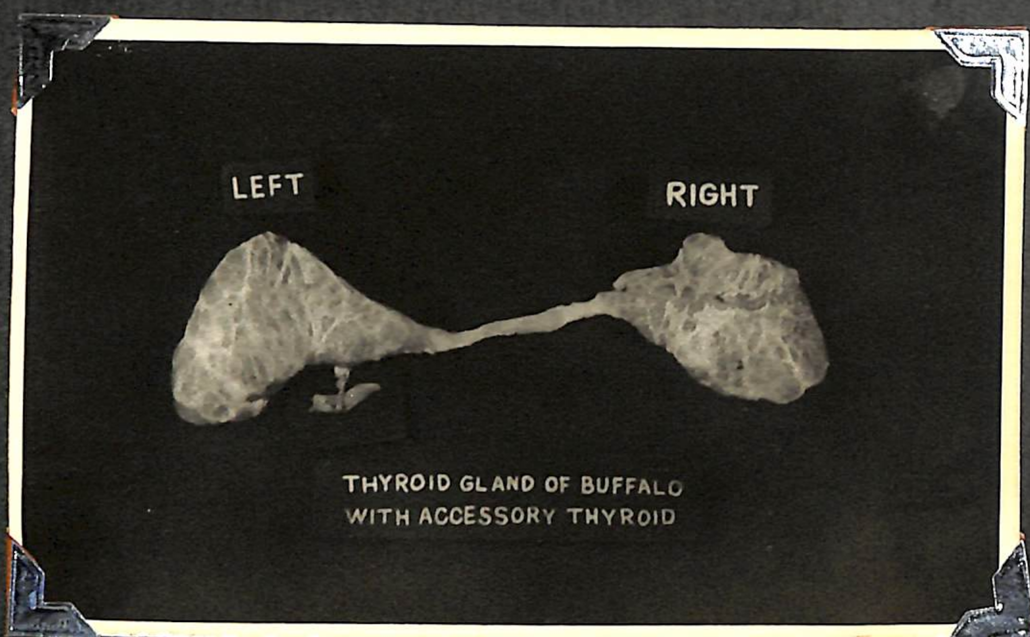
The lateral surface was related to sternocephalicus muscle, carotid artery, internal jugular vein and vagosympathetic nerve.

The medial surface was slightly cancave and was attached mainly to the trachea and partly to the cricoid cartilage. It was related to cricopharyngeus muscle, thyroid artery and recurrent laryngeal nerve.(

The average length, width, thickness and volume of the left thyroid lobe in young animals were 2.55 cm., 1.66 cm., 0.70 cm. and 2.44 ml. respectively, while that of the adult animals were 4.52 cm., 3.61 cm., 1.19 cm. and 11.30 ml. respectively (vide Table Nos. 1 and 2.)


LEFT
RIGHT
THYROID GLAND


LEFT
RIGHT
THYROID GLAND


LEFT
RIGHT
THYROID GLAND OF BUFFALO
WITH ACCESSORY THYROID

The average length, width, thickness and volume of right thyroid lobes of young animals were 2.06 cm., 1.66 cm., 0.84 cm. and 1.73 ml. respectively, while that of the adult animals were 3.82 cm., 3.45 cm., 1.17 cm. and 8.10 ml. (vide Table No. 1 & 2).

The total weight of the whole gland (Lobes and Isthmus) of young and adult were 4.20 gm. and 21.72 gm. respectively.

Isthmus was a band like structure which connected the lateral lobes. However, absence of Isthmus was observed in one adult female buffalo out of twenty experimental animals in the present investigation. Nineteen experimental animals had Isthmus which were in the form of a fibrous band with glandular tissue. The Isthmus was attached to the trachea laterally and venterally with pretracheal and deep cervical fascia. It was situated across the infero lateral face of the trachea usually inbetween 1st and 2nd tracheal rings. It was covered by sterno-thyrohyoideus muscle. Its average length, width and volume in young animals were 3.63 cm., 0.24 cm. and 0.74 ml. respectively while that of the adult animals were 7.16 cm., 0.58 cm. and 1.70 ml.

The description of the thyroid gland of Indian buffalo as presented above was in agreement with the description made by Raghawan (1964) in ox, Sisson and Grossman (1961) in cattle, Neal and Rand (1947).

The observation made regarding the level of Isthmus and its attachment to lateral lobes were similar to findings of Jones (1946) in man and Karski (1964) in monkey.

In this investigation, it was found that in 20 experimental animals 5% animal had separate lobes but Karski (1964) noted

14.7% separate lobes in monkey.

The size of the left thyroid was found to be larger than that of right one.

Last (1963) described the presence of pyramidal lobe in man. It was not observed in the present investigation.

Microscopic Anatomy

Capsule

The thyroid gland of Indian buffalo was enveloped in a capsule which was composed of three layers.

The outer layer was predominantly composed of collagen fibers with a few reticular and elastic fibers.

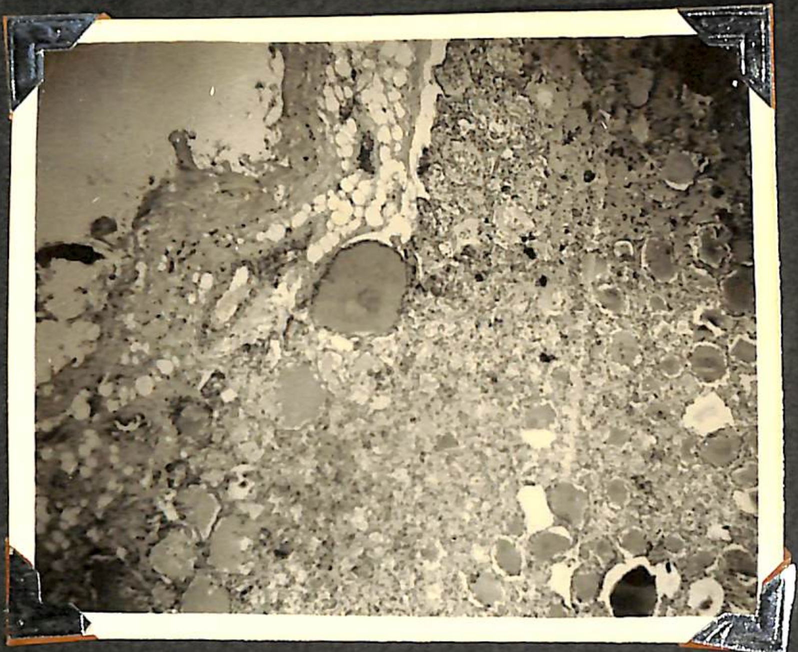
The middle layer was composed of adipose tissue entermingled with few bundles of collagen fibers. In some animals the middle layers was found to be absent.

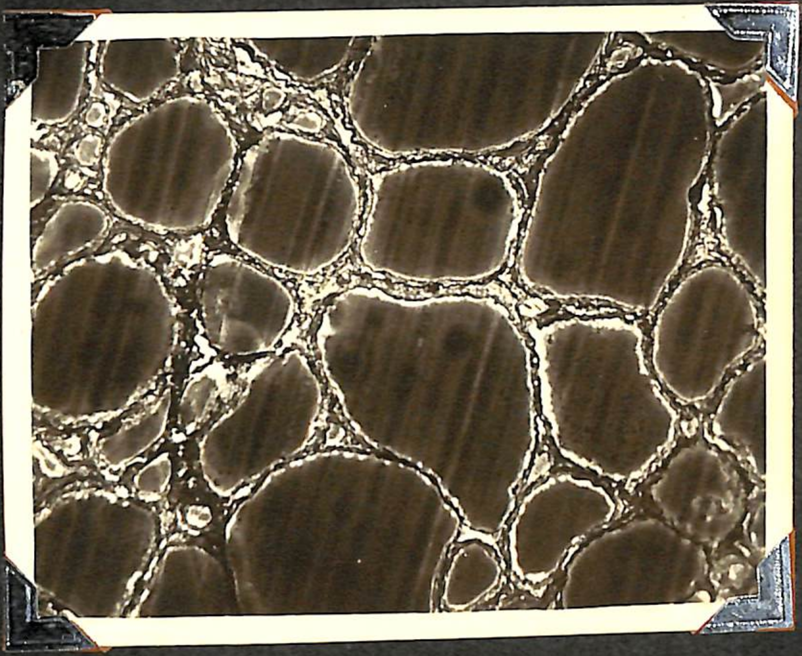
The inner layer was made up of collagen, reticular and elastic fibers.

The outer layer was composed of dense collagen fibers. It took red stain with Weigert and van Gieson stain and blue with Mallory connective tissue stain. These fibers were almost parallel to the outer surface of thyroid follicle. The elastic fibres stained deep purple with Gomori's aldehyde fuchsin stain. With Gridley's reticular stain reticular fibres were seen black.

The middle layer was composed of mainly of adipose connective tissue with few collagen fibers.

The inner layer of the capsule was closely related with follicles of the gland and were regarded as true capsule of the gland. It was mainly made up of collagen and reticular fibers.





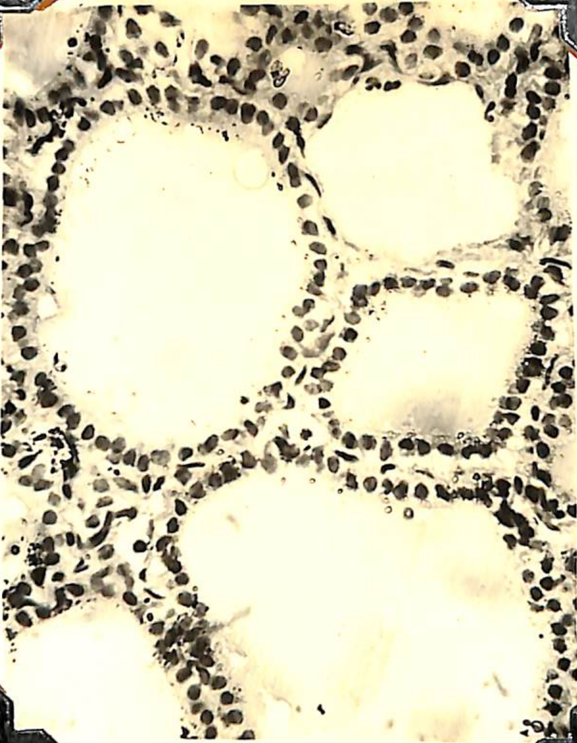


TABLE NO. 3A

"MALE BUFFALO"

(All measurements in μ)

Animal No.	Capsule thickness	FOLLICULAR DIAMETER					
		Round follicle		Oval follicle			
		Smallest	Largest	Smallest		Largest	
				Minor	Major	Minor	Major
1	140	28	56	70	148	154	224
2	280	56	114	28	56	196	224
3	420	112	140	42	56	280	420
4	240	28	112	70	182	224	350
5	540	28	210	28	42	126	210
6	420	28	56	84	140	462	560
7	420	70	112	56	70	378	532
14	140	56	280	42	70	210	350
15	420	28	112	42	70	98	140
16	280	42	112	42	56	140	210
Average	330	47.6	130.4	50.4	89.0	226.8	322.0
S.E.±	42.39	8.64	21.47	15.97	15.37	36.29	45.91

TABLE NO. 3B

"FEMALE BUFFALO"

(All measurements in /u)

Animal No.	Capsule thickness	FOLLICULAR DIAMETER					
		Round follicle		Oval follicle			
		Smallest	Largest	Smallest		Largest	
				Minor	Major	Minor	Major
8	1120	112	420	252	392	630	840
9	1120	148	630	140	148	350	490
10	280	70	210	42	70	378	532
11	280	28	112	56	140	569	728
12	140	28	350	56	70	504	602
13	140	42	210	84	140	140	518
17	56	28	280	148	252	140	420
18	252	112	420	70	98	280	656
19	56	148	630	252	148	350	602
20	560	210	252	42	70	210	364
Average	400.4	92.6	351.4	114.2	152.8	354.2	575.2
S.E.±	128.4	2.0	86.5	25.7	31.7	51.5	45.0

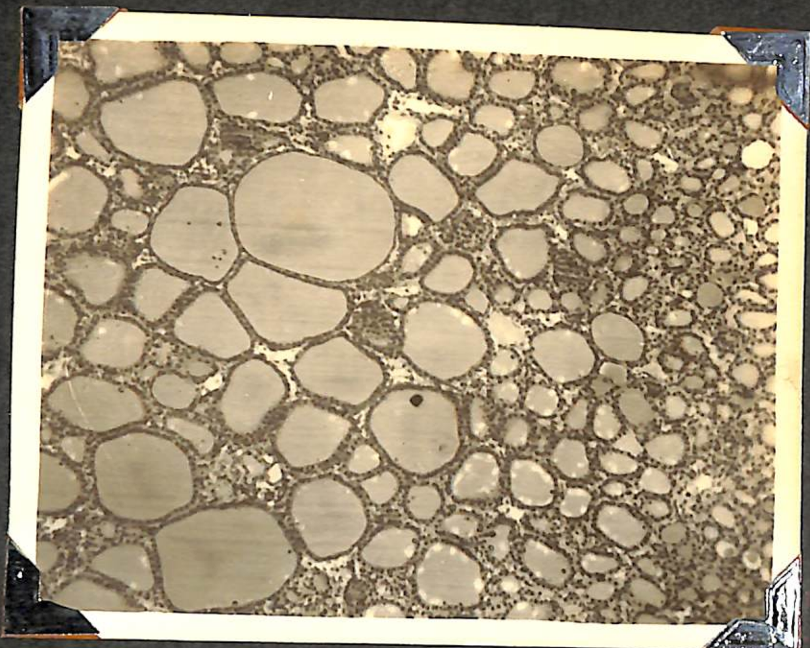
The average thickness of the capsule was 330 /u in young animals while 400.4 /u in adult. No smooth muscle fibers were found in the capsule. The capsule was highly rich in blood vessel and nerves, which could be seen as transverse or longitudinal cut sections (Plate No. V).

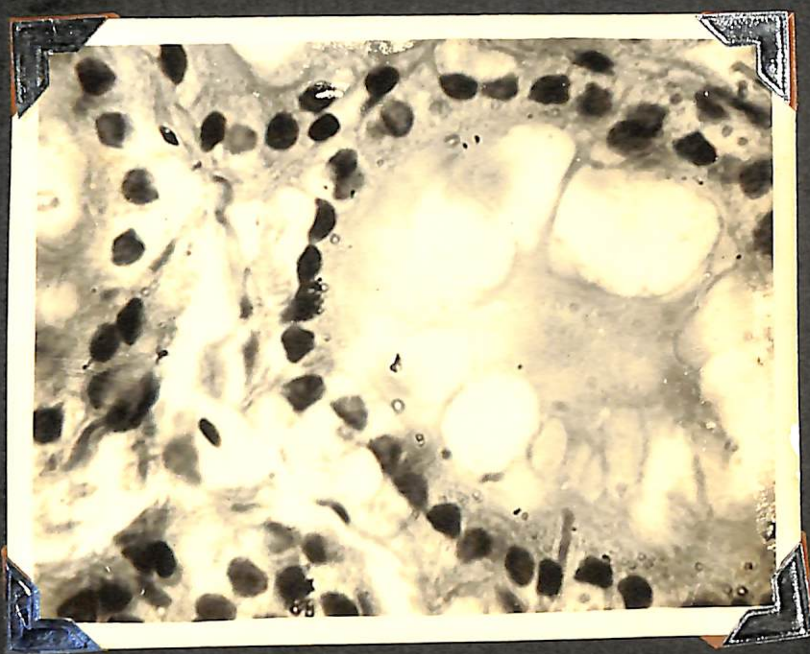
The collagen and reticular fibers constituted the main component of the fibroarchitecture of the thyroid gland. The cellular component of the stroma was very less and was mainly comprised of fibroblast cells with elongated nuclei. The Fibrous septa were seen descending into the gland parenchyma which besides forming the thick stromal septum circumscribed the thyroid follicle like a chicken wire net (Plate No. VI).

Das <u>et al.</u> (1965) found collagen fibers, reticular fibers and a few smooth muscle fibers into the capsule of thyroid gland in bull and bullock. Roy (1971) did not found the presence of smooth muscle fibers in the thyroid capsule of buffalo. In present investigation smooth muscle fibers were not observed in the capsule of the thyroid gland in Indian buffalo. Maximow and Bloom (1952), Arey (1968) in man Trautmann and Fiebiger (1957) in domesticated animals described that thyroid capsule was made up of connective tissue. As such the present findings were almost in perfect agreement with the findings of different research workers and authors inumerated above except Das <u>et al.</u> (1965).

Follicle

Thyroid gland of Indian buffalo was made up of numerous follicles and small amount of interfollicular stroma. These





follicles were filled with secretion product called colloid. Hence each follicle was not only structural unit but also functional unit of the gland. The shape of the follicle varied greatly. Most of them were oval while a few were round and irregular.

In young experimental animals the diameter of round follicle varied from 47.6 /u to 130.4 /u. In case of smallest oval follicle the minor diameter was 50.4 /u and major was 89.0/u. In largest oval follicle the minor diameter was 226.8 /u and major 322.0 /u.

In adult experimental animal the diameter of the round follicle varied from 92.6 /u to 351.4 /u. In case of smallest oval follicle the minor diameter was 114.2 /u and major 152.8 /u. In largest oval follicle the minor diameter was 354.2 /u and major 575.2 /u.

The variation in the size of the follicle was due to the fact that the different irregularly arranged follicles were cut at a different plane in a particular section. It was ascertained on observing the serial sections cut at 15 /u thickness. Further it was also noticed that when the follicles were cut tangentially, it appeared as a clump or aggregation of the cells.

In young animals the follicles were found to be smaller than those of the adult. The size of the follicles varied according to the activity of the gland. It was found that inactive follicles were larger and active follicles were usually smaller in both young and adult animals.

In present investigation it was found that the large follicles predominantly appeared in deeper zone of the gland.

However such arrangement was not observed in a few animals.

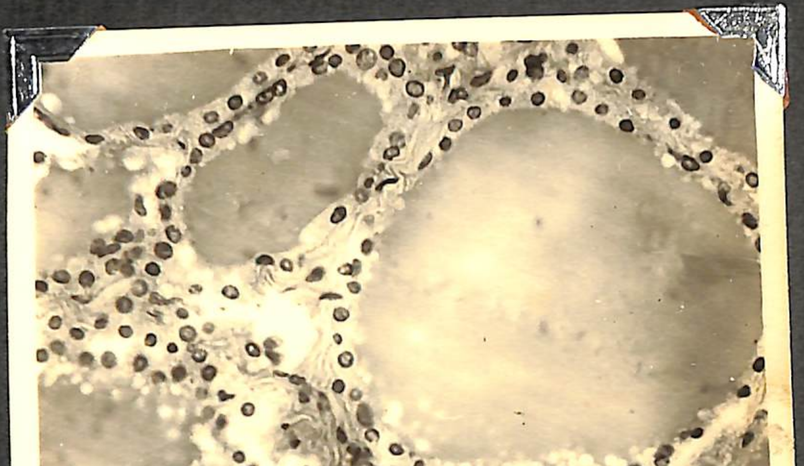
Examination of P.A.S. and haematoxylin stained sections of the thyroid gland showed that follicles had purple basement membrane which surrounded the follicle completely.

Tomonari (1959) in mammal, Yasunobu *et al.* (1966) and Mosier *et al.* (1967) in rat noted that smaller follicles were present in deeper area of the gland and larger one in the periphery. Das *et al.* (1965) in bull and bullock, Roy (1971) in buffalo found that smaller follicles were mostly located towards the periphery and larger one in the center. The present findings were just reverse of the observations made by Tomonari (1959), Yasunobu *et al.* (1966) and Mosier *et al.* (1967) but tallied with findings of Das *et al.* (1965) and Roy (1971).

Isler *et al.* (1968) in rat noticed that in some places the base of the epithelium were in direct contact with the base of the epithelium of another follicle. They further cited that Yagizawa (1956) reported an incomplete wrapping of the follicle by C.T. in several other species (rabbit, guineapig, hamaster, cat, dog, pig, horse, cattle, monkey). But in present investigation it was found that basement membrane and C.T. was constantly present inbetween the follicles.

Follicular cell

Follicles were lined either by simple squamous or simple cuboidal or simple columnar epithelium. These follicular cells were based upon P.A.S. positive basement membrane. The follicular epithelium was made up of two types of cell — Follicular cells proper and Light cell.



The size and shape of follicular cells were in consonence with physiological and functional activities of the follicles. It was found that in active follicles the follicular cells were of high cuboidal or columnar type whereas in inactive follicles these cells were of low cuboidal or even squamous. It was also observed that in certain follicles, some cells were squamous, some were cuboidal and some were columnar. Similar observations were made by Roy (1971) in buffalo.

Each cell had large basophilic nucleus. The columnar and cuboidal follicular cells had spherical or oval nuclei; whereas the squamous follicular cells contained flattened elongated nuclei which were hyperchromatic and thus their nuclear detail were not distinct. The spherical and oval nuclei occupied the greater portion of the cell cytoplasm. They were vasiculated with diffuse chromatin materials. They contained one or more nucleoli. The spherical and oval nuclei were usually situated in the center or towards the basement membrane; whereas the nuclei of squamous follicular cells were situated upon basement membrane. The central location of the large spherical nuclei in the follicles lined with cuboidal epithelium in the thyroid glands under present investigation were in agreement with the observation made by Das et al. (1965) in bull and bullock. The present findings on the whole were in perfect conformity with those of Roy (1971) in buffalo.

The nucleus was found to be Feulgen reactive which indicated the presence of D.N.A. Roy (1971) made similar observation in the buffalo (Plate XXIII). The mitotic figures were found among the follicular cells. Stux et al. (1961) also observed mitotic figures in thyroid gland of rat (Plate XX).

The columnar follicular cells had slightly basophilic cytoplasm. The supra nuclear zone of cell had more basophilic cytoplasm. The infra nuclear zone was usually vacuolated but contained some basophilic cytoplasm also. (Plate IX). The cytoplasm was found basophilic specially when the follicle were active. When stained by Mallory stain (the aniline blue and orange G technique) the intracellular colloid droplets took either blue or yellow stain. The P.A.S. positive granules were also observed at the apical border's of the follicular cells. The above observation were almost in agreement with the findings made by Roy (1971) in the thyroid gland of buffalo. Cytoplasm showed the presence of thin rod like mitochondria. They stain red by Metzner's method.

The cell boundaries of some follicular cells were quite distinct but the cells with indistict cell boundaries were also not uncommon. The terminal bar's were seen in columnar and cuboidal follicular cells.

Trautmann and Fiebiger (1957) in domesticated animals, Copenhaver (1964) in man, Shin (1965) in bovine, Roy (1971) in buffalo also observed the presence of terminal bar's among the follicular cells.

The distinct cell boundaries as observed in present investigation were in perfect agreement with the observations made by Harrison et al. (1962) in common seal and Copenhaver (1964) in man.

Tomonari (1959) in cow, pig, horse, cat, dog, rabbit, rat and guineapig; Copenhaver (1964) and Bloom and Fawcett (1968) in man described that in general the epithelium tended to be squamous when the gland was underactive and were not active in

colloid formation, while columnar when the gland was overactive. These findings tallied with the observations made in present investigation in buffalo.

In young animals the average height and width of squamous follicular cell was found to be 4.13 /u and 7.85 /u, the cuboidal follicular cell was 6.43 /u and 6.55 /u and columnar follicular cell was 9.52 /u and 5.32 /u. The average diameter of nucleus was found to be 3.48 /u.

In adult female buffalo the average height and width of squamous follicular cell was 4.30 /u and 8.06 /u, the cuboidal follicular cell was 6.75 /u and 6.86 /u and columnar follicular cell was 10.45 /u and 6.09 /u. The average diameter of nucleus was found to be 4.04 /u.

Bhatnagar (1955) in buffalo noted 3.69 /u average height of follicular cell in summer, 3.09 /u in autum, 4.47 /u in winter and 4.79 /u in spring. Harrison et al. (1962) recorded 6.36 /u as mean cell height of the follicular cell in common seal.

Light cell

In addition to the principal cell i.e. the follicular cell proper, there was another type of cell present in the follicular epithelium best known as "Light cell". Although it has been given many names by different workers, such as "Parafollicular cell", Nonidez ([illegible], 1932); "Mitochondria" rich cell", Bloom and Fawcett (1968), "C cell" (Pearse 1966, Carvalheira and Pearse 1967; Matsuzawa and Kurosumi 1967; Beskid and Roseizewska 1968) and "Light cell" (Stux et al. 1961; Sarkar and Isler 1963; Amat and Vazquez 1965; Halmi 1966; Young et al. 1968). The name "Light cell" was considered to be the best and hence adopted under the present investigation.

Nonidez (1932; Pearse 1966; Bussolati Pearse 1967; Steven et al. 1970) in dog, (Stux et al. 1961; Sarkar and Isler 1963; Matsuzawa and Kurosumi 1967) in rat, (Gabe (1961) in birds, reptiles and Amphibia, Amat and Vazquer (1965) in guineapig, (Amat and Garcia-Lacrew (1966; Carvalheira and Pearse (1967; Young et al. 1968) in pig, Kameda (1968) in cat, rabbit and mouse, Beskid and Roseizewska (1966) in man, ox, (Tashiro (1964; Stoekel 1967; Shively & Sow 1969) in mammals also noted the presence of second epithelial component in thyroid glands of respective animals.

The light cells were seen either singly or in a group of two or three. They could be readily identified in a section stained by Haematoxylin and eosin. The light cell had large nucleus with faintly stained cytoplasm. They were larger than those of follicular cell proper. Their shape and size varied greatly. They did not possess distinct cuboidal or columnar outlines. They were devoid of uniform contours and terminal bars. Sometimes they had smooth circular and oval outline. Occasionally they were polygonal and spindle shaped. The above findings were in agreement with findings of Stux et al. (1961) in rat.

The nuclei of light cells were round and were usually larger than those of the follicular cells proper and often contained vacuoles. Mitotic figures were not observed. The nuclei were eccentric but regular in outline. When stained with Cajal stain and Weil Davenports stain the nuclei were usually not seen and Vazquez 1965; Halmi 1966; Young et al. 1968). The name "Light cell" was considered to be the best and hence adopted under the present investigation.

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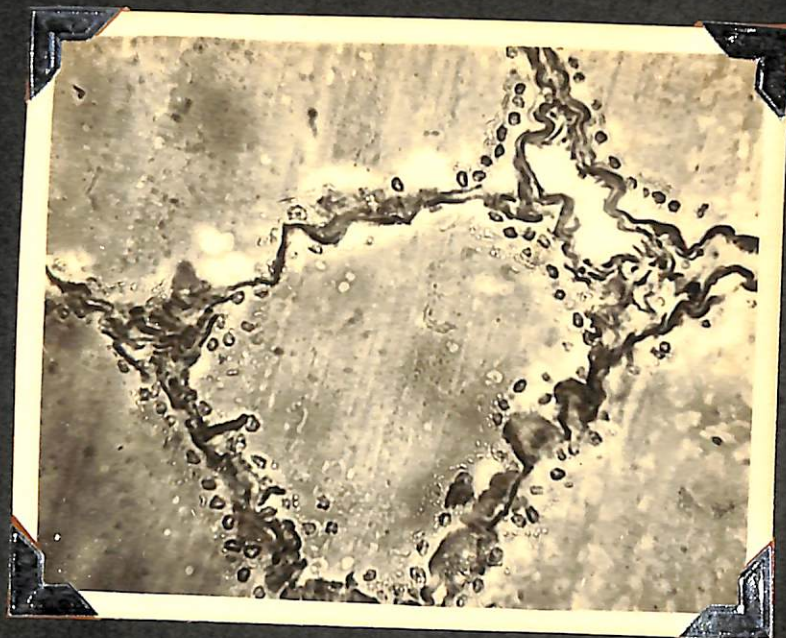

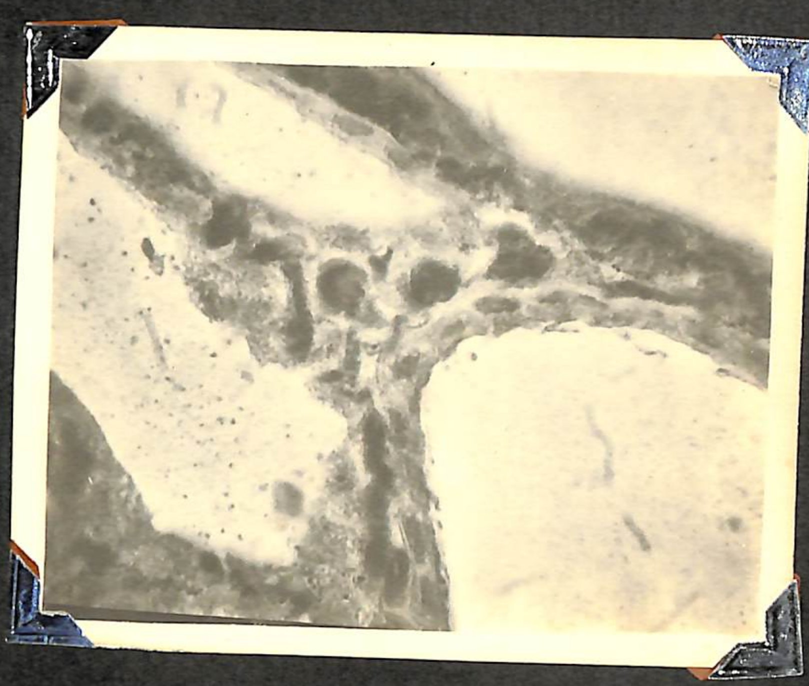

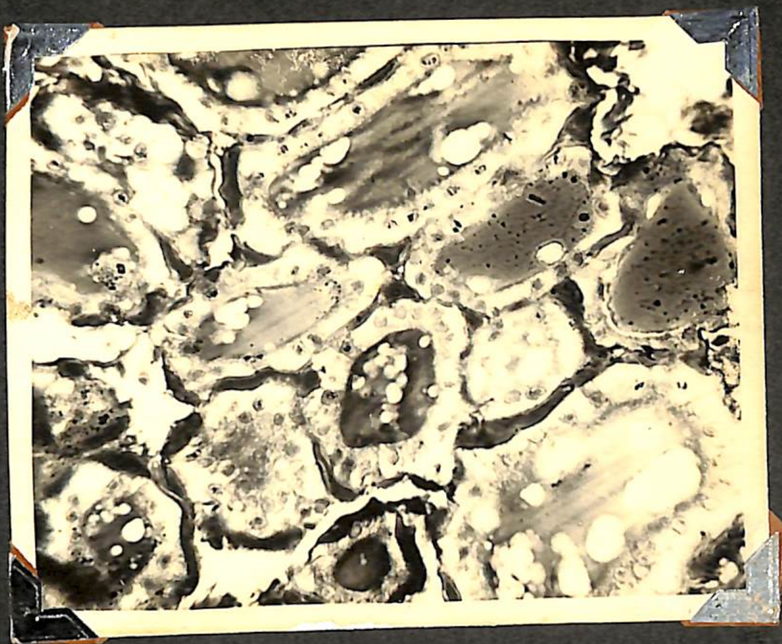

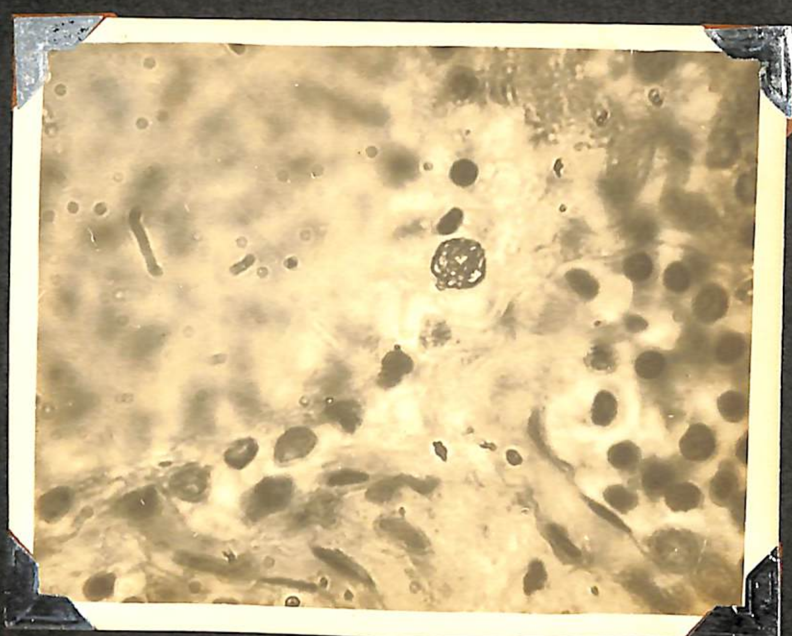

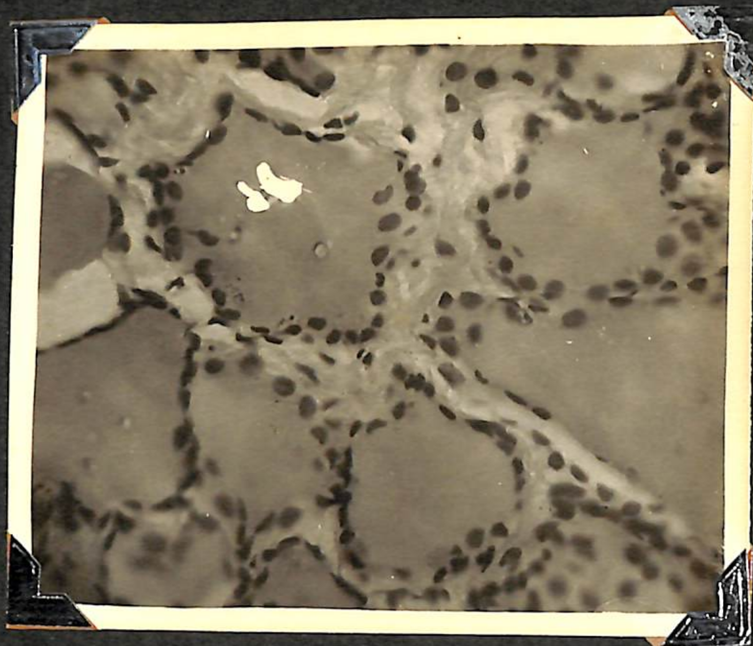

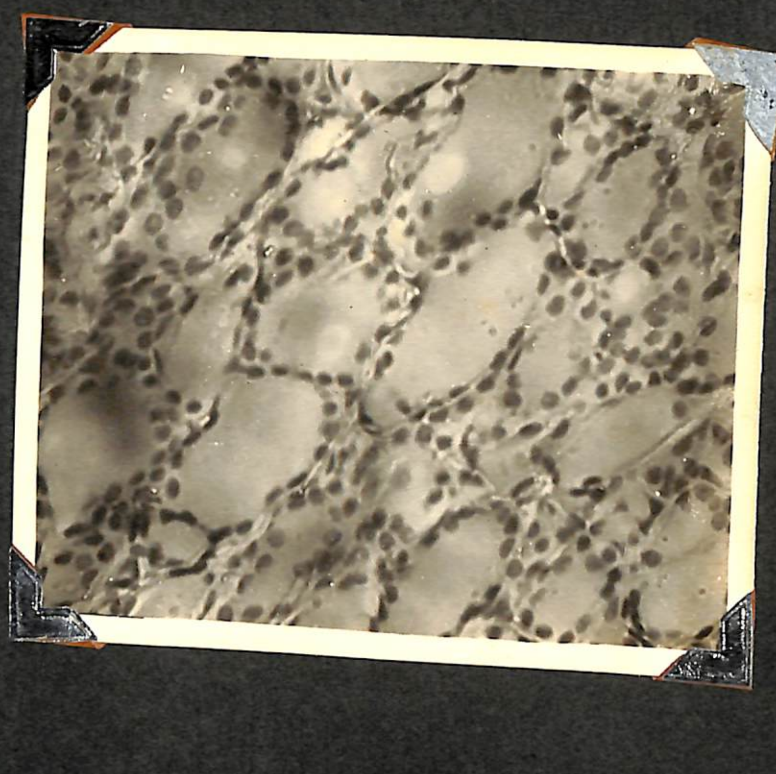

glandular tissue of the Isthmus resembled with the glandular tissue of the lateral lobes. No significant histological differences were noticed in the Isthmus of the thyroid gland under present investigation. As such it did not command a separate description of its own. However apparent differences were observed with the naked eye which have been already discussed along with the gross description of the gland.

<u>Accessory thyroid</u>

In one adult buffalo a glandular thyroid like mass was found just ventral to the trachea and very close to the thyroid gland (Plate IV). It was covered by a fibrous capsule. It contained the follicles of different sizes and had both active and inactive follicles. These follicles more or less resembled with the follicles of thyroid gland proper.

Trautmann and Fiebiger (1957) described the presence of accessory thyroid gland in domesticated animals. According to them they have similar structure and function as the regular thyroid. Karski (1964) found 4.4% accessory thyroid gland in monkeys. But in the present investigation only 5% accessory thyroid gland was noted in Indian buffalo. Sanson (1967) also recorded the presence of accessory thyroid gland in goat.

The microscopical structures of accessory thyroid observed under the present investigation were similar to the description made by Trautmann and Fiebiger (1957) in domesticated animals. The percentage of occurrence of accessory thyroid recorded in the present investigation however differed by 0.6 percent from that of Karski (1964) in monkey.

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Trautmann and Fiebiger (1957) described that blood vessels were numerous and capillaries from network which surrounded the follicles in the thyroid gland of domestic animals. Ivanova (1962) in human noticed that outer zone where the main arterial stem and major branches occurred and the central zone where the arterial vessels were of smaller diameter. Cecio and Caputo (1963) noted the presence of thyroid blocking structures in blood vessels made up of endothelial cells, valves and myoepithelial cells in human ... bovine and ovine thyroid gland.

Observations ... similar to those of Trautmann and Fiebiger (1957) and Ivanova (1962) were also noticed in the thyroid gland of Indian buffalo. However, the thyroid blocking structures in the human bovine and ovine thyroid as observed by Cecio and Caputo (1963) could not be ascertained under present investigation.

Nerve supply

The th... and of Indian buffalo was richly innervated. These nerve fibers were in close association with the blood vessels. Both the cross and longitudinal cut sections of nerve bundles and nerve fibers could be seen in the stroma. These nerve bundles were seen red with thionin stain. In silver impregnation method of Cajal these nerve bundles took black stain. They were present all along the stroma around the individual follicle. Solitary ganglion cells were also observed in the thyroid stroma.

Trautmann and Fiebiger (1957) in domestic animals described that nerve bears ganglion supplied fibers to the follicular epithelium. Okamato (1959) noted the presence of myelinated fibers immediately adjacent to follicle in thyroid gland of man and dog. Kladienko (1962) in cattle and Sarrat (1965) in rat noted the presence of ganglion cell in connective tissue of thyroid gland.

Observation made under present investigation tallied with those of Trautmann and Fiebiger (1957) , Kladienko (1962)in cattle and Sarrat (1965) in rat.

HISTOCHEMISTRY

P.A.S. positive materials

The present investigation section of thyroid glands from different animals were stained by periodic acid schiff method to study the distribution of polysaccharides and complex compounds with protein and Lipids. The follicular cells showed weak positive reaction whereas connective tissue moderately and colloid were strongly reactive with P.A.S. The light cells were devoid of any P.A.S. positive material (Plate X).

When human saliva was allowed to act on a section the colour of P.A.S. reactive material stained faintly. This was probably due to removal of glycogen from the sections.

Belanger and Bois (1960) in guineapig, Mosier and Richter (1967) and Seljelid (1967) in rat noted that intracellular and intrafollicular colloid were P.A.S.positive, observation made by these authors tallied with observations made under present investigation.

The follicular cells and colloid showed mild positive for acid mucopolysaccharides with Alcian blue stain. Bollet et al. (1959) in dog and Roy (1971) in buffalo were made similar observation in the thyroid gland of buffaloes. In this stain the distinction between light and follicular cell could not be ascertained.

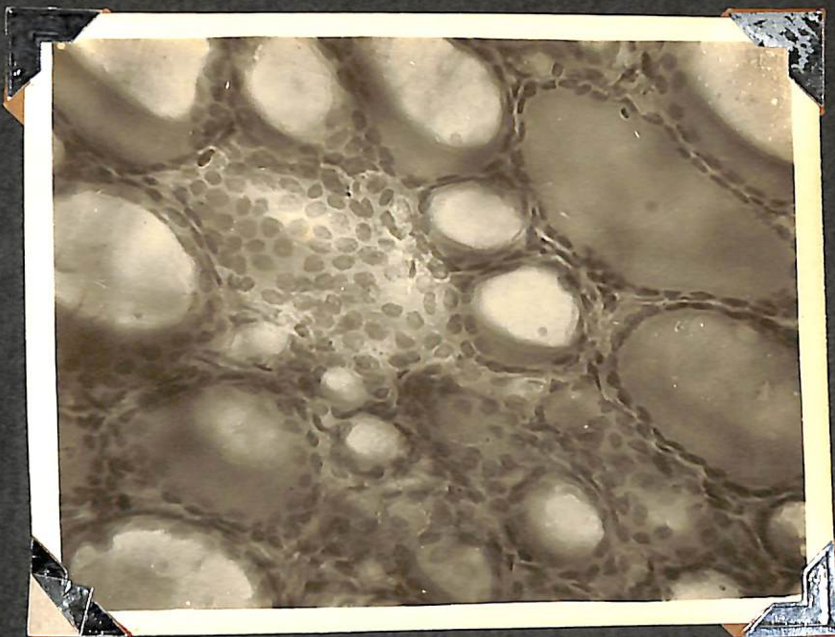
When stained by Best's carmine stain, the follicular cell connective tissue and colloid showed weakly reactive for glycogen. The light cells were negative for glycogen. Copenhaver (1964) in man described that glycogen was present in form of glycoprotein and thyroid gland.

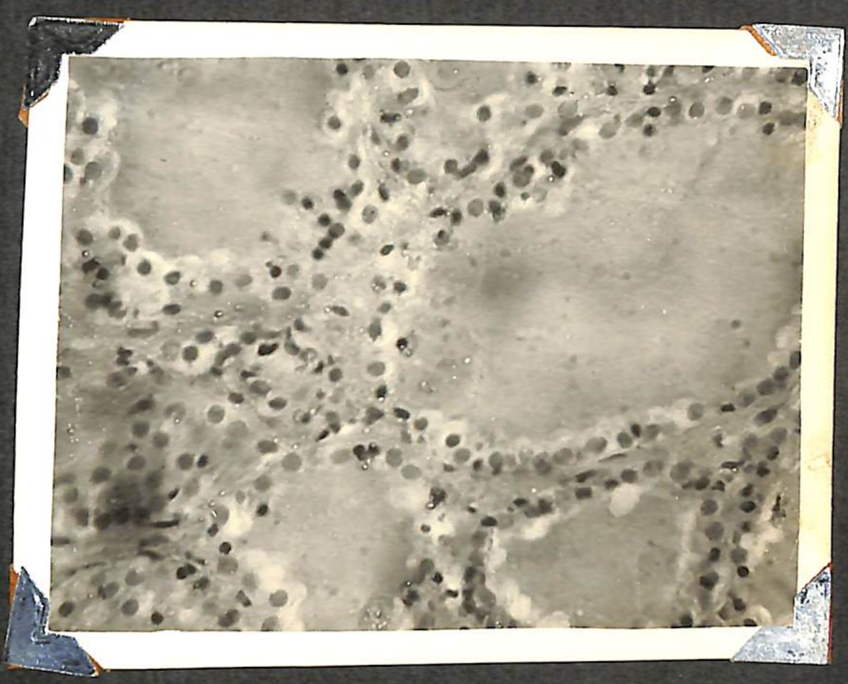
Protein

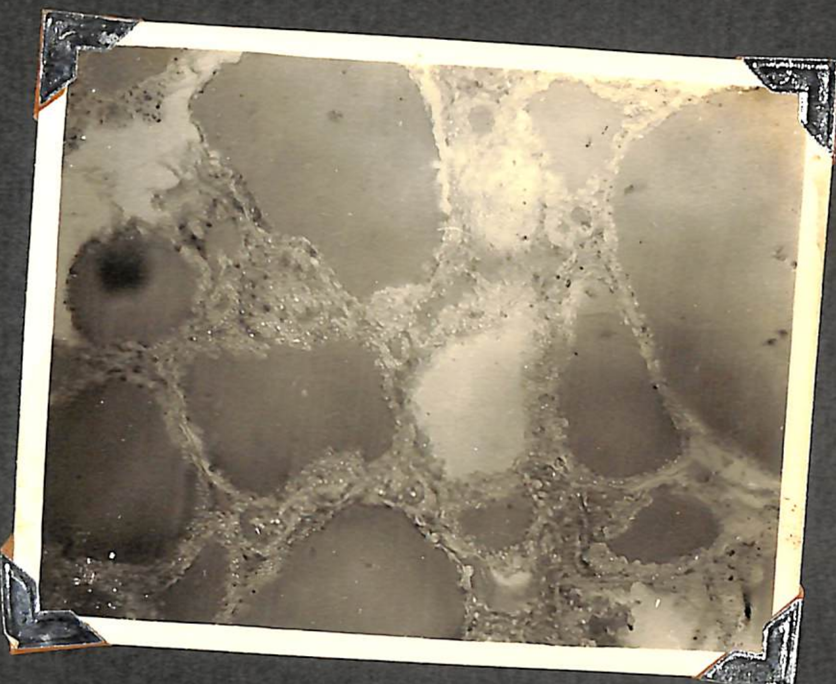
When sections were stained by bromphenol blue method the follicular cells and connective tissue showed moderately positive reaction. The light cells showed weakly positive and colloid showed strongly positive for protein.

Robbins et al. (1959) noted the presence of iodoprotein in normal human and sheep thyroid tissue Feyrter et al. (1964) observed the presence of lipoprotein in human thyroid.

Belanger and Bois (1964) noted that the colloid of rat thyroid







biologically active aminoacid.

Similar observations were made under present investigation. Some follicle took blue colour, some took orange colour, while a few showed tinctorial differences. The marginal colloid were of blue colour while the colloid of deeper part took yellow colour or reverse the same in follicle which showed tinctorial differences (Plate XVI). The above findings were in agreement with the findings made by Roy (1971) in buffalo.

Lipids

Thick Frozen sections were stained for lipids by Sudan IV and Isopropanol oil red o method. No lipids were observed either in follicular cells, or colloid in thyroid gland of buffalo under present investigation.

Calcium and Iron

Histochemical observations for Calcium and Iron were also made. However, their presence could not be revealed in the thyroid gland of Indian buffalo under present investigation.

SUMMARY AND CONCLUSION

1. Histological and histochemical studies were made on thyroid glands of Indian buffaloes. The thyroid glands of twenty (ten young male and ten adult female buffaloes) were collected and preserved in various preservative such as 10% formalin, neutral aqueous formalin, buffer formalin, Bouin's Fluid, Heidenhain's 'Susa', and Helly's Fluid. Thin sections of 3 to 5 /u thickness were cut for histological studies whereas thick sections of 10 to 15 /u thickness were cut for histochemical investigation. Some formal fixed frozen section at 10 /u thickness were also cut for lipids.

2. The following histological stains were used in present investigation :

(i) Harris's haematoxylin and eosin used as routine method of staining.

(ii) Gomori's aldehyde fuchsin for elastic fibers.

(iii) Azocarmine mallory stain for C.T. and colloid.

(iv) Gridley's reticulum stain for reticular fibers.

(v) Weigert and van Gieson stain was employed for collagen fibers and muscle fibers.

(vi) Thionin for nerve tissue and nerve tract.

(vii) Mallory's stain for connective tissue.

(viii) Metzner's method for mitochondria.

The following histochemical stains were used in present investigation :

(i) Periodic acid-schiff's stain for P.A.S. positive materials.

(ii) Best's carmine for glycogen.

(iii) Alcian blue methods for acid mucopolysaccharides.

(iv) Feulgen reaction for D.N.A.

(v) Von Kossa's method for calcium deposits.

(vi) Perls' method for ferric iron.

(vii) Mercury baromphenol blue method for protein.

(viii) Ninhydrin-Schiff method for protein bound NH_2.

(ix) Millon reaction for Tyrosine.

(x) Sudan IV and Oil O red method for lipids.

(xi) Aniline blue and orange G technique for iodinated and non-iodinated thyroglobulin.

Stains used for light cells :-

(i) Weil-Davenport's technique

(ii) Cajal stain

(iii) Heidenhain's azan stain

3. The average length, width, thickness and volume of the left thyroid lobe in young animals were 2.55 cm., 1.66 cm., 0.70 cm. and 2.44 ml. respectively, while that of the adult animals were 4.52 cm., 3.61 cm., 1.19 cm. and 11.30 ml. respectively. The average length, breadth, thickness and volume of right thyroid lobe of young animals were 2.06 cm., 1.66 cm., 0.84 cm. and 1.73 ml. respectively, while that of adult animals were 3.82 cm., 3.45 cm., 1.17 cm. and 8.10 ml. The total weight of the whole gland of young and adult were 4.20 gm. and 21.72 gm. respectively. The size of the left thyroid lobe was found to be larger than that of right lobe.

4. Absence of Isthmus was observed in one adult female out of 20 experimental animals. Its average length, width and volume in young animals were 3.63 cm., 0.24 cm. and 0.74 ml.respectively

while that of the adult animals were 7.16 cm., 0.58 cm. and 1.70 ml.

5. Thyroid gland was enveloped by C.T. capsule which was made up of following layers :

a) The outer layer was predominantly composed of collagen fibers with a few reticular and elastic fibers.

b) Middle layer was composed of adipose tissue intermingled with few bundles of collagen fibers. However absence of this layer was noticed in some animals.

c) The inner layer was made up of collagen, reticular and elastic fibers.

The average thickness of the capsule was 330 μ in young animal while 400.4 μ in adult. It was highly rich in blood vessels and nerves. No smooth muscles were observed in the capsule.

6. The gland was made up of numerous follicles and small amount of interfollicular stroma. In young experimental animals the diameter of the round follicle varied from 47.6 μ to 130.4 μ. In case of smallest oval follicle the minor diameter was 50.4 μ and major 89.0 μ. In largest oval follicle the minor diameter was 226.8 μ and major 322.0 μ.

In adult experimental animals the diameter of round follicle varied from 92.6 μ to 351.4 μ. In case of smallest oval follicle the minor diameter was 114.2 μ and major 152.8 μ. In largest oval follicle the minor diameter was 354.2 μ and major 575.2 μ. Each follicle was separated by connective tissue and had its own basement membrane. The larger follicles predominantly appeared in deeper area of the gland. When the follicles were cut tangentially, it appeared as a clump or aggregation of cells.

7. Follicles were lined either by simple squamous or simple cuboidal or simple columnar epithelium. The size and shape of follicular cells depended upon the physiological and functional activity of the gland. They had basophilic cytoplasm and contained either spherical, oval or flattened elongated nuclei. The Nucleus was strongly feulgen reactive. The terminal bars were seen among the follicular cells. In young animals the average height and width of squamous follicular cell was found to be 4.13 /u and 7.85 /u, the cuboidal follicular cell was 6.43 /u and 6.55 /u and columnar follicular cell was 9.52 /u and 5.32 /u. The average diameter of nucleus was found to be 3.48 /u. In adult female buffalo the average height and width of squamous follicular cell was 4.30 /u and 8.06 /u, the cuboidal follicular cell was 6.75 /u and 6.86 /u, and columnar follicular cell was 10.45 /u and 6.09 /u. The average diameter of nucleus was found to be 4.04 /u.

8. The mitotic figures were found among the follicular cells.

9. Light cells were seen among the follicular cell proper. They constituted the second epithelial components of thyroid gland. They were seen either w singly or in a group of two or three. Light cell had larger nucleus with faintly stained cytoplasm. They were devoid of uniform contours and had many shapes. These light cells were located among the droplet laden follicular cells or between these cells and basement membrane. They were separated from direct contact with colloid by whole of follicular cell or by a layer of its cytoplasm. Terminal bars were not observed inbetween light cells. The light cells showed argyrophilia when stained with

Cajal and Weil Davenport's stains. By Mallory stain and Heidenhains azan stain the cynophilic secretory granules of these cells stained blue and occasionally red. Nuclei were eccentric but regular in outline. Mitotic figures were not observed. In young animals the average height, width of the light cell and diameter of nucleus was found to be 12.44 /u, 10.45 /u and 4.63 /u respectively, whereas in the adult the average height, width of light cell and diameter of the nucleus was found to be 11.91 /u , 10.12 /u and 4.09 /u, respectively. These light cells were supposed to secret a harmone the thyrocalcitonin.

10. Each follicle contained a viscous homogenous fluid the colloid which was the secretion product of follicular cell. They often contained desquamated follicular cells. The quantity of colloid depend upon the physiological, activity of follicles. In the active follicle it was thiner and less viscous while in inactive follicle it was thick and more in quantity. The colloid was found to be basophilic in active follicle while the colloid of inactive follicle showed acidophilia. Vacuoles and irregular spaces were seen inbetween the colloid and epithelium. Vacuoles were seen predominantly in active follicles.

11. The Isthmus was a glandular band connecting the lateral lobes. No significant histological differences were noticed in the Isthmus than those of the lateral lobes of the thyroid gland.

12. In the vicinity of one of the thyroid gland, one small accessory thyroid was found. The microscopical structure of the accessory thyroid observed under the present investigation were similar to those of thyroid gland proper.

13. The thyroid gland was richly supplied with blood vessels, and nerves. These blood vessels and nerves were seen in the stroma. Solitary ganglionic cells were observed in the thyroid stroma.

14. Follicular cells were P.A.S.positive, they were also positive for glycogen, acidmucopolysaccharide, protein, tyrosine, reactive protein bound NH_2 , whereas negative for lipid, calcium and iron. Light cells were positive for protein and were P.A.S. negative. They were also negative for protein bound NH_2 and glycogen.

15. The colloid was P.A.S. positive and was also positive for protein, tyrosine, reactive protein bound NH_2, glycogen and acidmucopolysaccharides whereas negative for lipid, calcium and iron.

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A THESIS

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[illegible] fulfilment of the requirements

for the degree of

[illegible] OF SCIENCE (Veterinary)

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