

## HISTOLOGICAL STUDY OF THE THYROID AND PARATHYROID GLANDS IN IRAQI BUFFALO "*BUBALUS BUBALIS*" WITH REFERRING TO THE SEASONAL CHANGES

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### ABSTRACT

The present study included the collection of thyroid and parathyroid glands from the abattoirs of middle of Iraq in Summer and Winter seasons 2006-2007. The samples were fixed by 10% formalin and processed by the routine histological techniques, then stained by H and E, PAS and Van Gieson stain, then studied under light microscope. Dimensions were measured by ocular micrometer. Analysis of data was done by T-test.

The most striking result declared that the thyroid gland showed no differences in histological characters between both seasons as the buffalo was poor thermoregulator animal. It is tempting to speculate that the thyroid gland provide an adaptive advantage for survival of the buffalo in the nature arid environment. The parafollicular cells (C-cells) were sparse and found singly in the thyroid glands. Flat dark nuclei of myoepithelial cells were recognized around the thyroid follicles. This study was referred to the presence of accessory thyroid gland which contains simple follicular epithelium, and little amount of the colloid. Our current study revealed the presence of internal and external parathyroids. The oxyphilic cells in the internal parathyroid gland were not observed.

**Note:** this work in part of Master Science Thesis, submitted to the College of Veterinary Medicine, Baghdad University.

### INTRODUCTION

Among the endocrine glands, adrenal and thyroid respond quickly to the changes in the environmental condition and thus bring about the necessary physiological adjustment to the changing environment (1 and 2). Hyperthyroidism causing increased metabolism and sweating (3 and 4). Furthermore, (5) stated that thyroid influence the rate of heat production. This action of thyroid hormones allows the animal to adapt to the variation in environmental temperature. Thyroid hormones is involved in thermoregulation of the body. In collaboration with other hormones, it elevate body temperature by oxidant of fat, CHO and protein and release heat (6 and 7).

Thyroid gland showed seasonal variation in weight, quantity of colloid and follicular epithelial height, suggesting to be inactive during quiescence and Winter dromency and active during time of recrudescence and breeding time similarly (8). When the gland is inactive, the colloid is abundant, the follicles are large and the cells which lining them are flat. When the gland is active, the follicles are small, the cells are cuboidal or columnar and the edges of the colloid is scalloped forming many small reabsorption lacunae (9 and 10). Similarly (11 and 12) have described the size of the follicles of thyroid gland in man during hypoactivity to be enlarged. When the colloid is strongly basophilic, the follicle containing it's in a stage of intense metabolic activity, as opposed to follicles containing acidophilic colloid (12).

The parafollicular cell (C-cell) produced the hormone thyrocalcitonin which act directly on osteoclast to decrease bone resorption which lower the blood calcium level, therefore, the effect of thyrocalcitonin antagonize that of parathormone (7, 13 and 14). Other authors have reported that C

cells were quite abundant in the thyroid of hibernators especially in prehibernating (fall) as attempt to reserve granular for the Winter (15, 16 and 17), while (18) have showed that in Summer the C-cells, appear, to be larger and more active. (19) Mentioned that C-cells were reduced the concentration of Ca in blood by inhibiting bone resorption.

(9) Found that the principle role of parathyroid hormone was to maintain the normal levels of Ca and phosphorous in the blood by inducing and increase absorption of calcium from the intestine and decrease the loss of Ca from the urine and resorption of Calcium from the bone (activation of osteocalasts).

Many authors have divided the parenchymal parathyroid cells into: chief cells of various functional stages, atrophic cells, multinucleated syncytial cells and oxyphilic cells (20, 21 and 22). Other authors classify the parathyroid cells according to the presence of fat cells or glycogen as active secreting and inactive resting (7, 23, 24, 25 and 26).

(27) Stated that oxyphilic cells do not appear in man until near the first decade of life. (10) found that the salmon calcitonin is of interest because it is more than 20 times active than human calcitonin is. From a review of the literature, it became clear that there is a paucity of the seasonal studies on the thyroid and parathyroid glands, therefore, this study was undertaken to focus light on this aspect.

## MATERIALS AND METHODS

Thyroid and parathyroid glands from twenty-six (right and left) sexually mature apparently healthy buffaloes slaughtered at local abattoir were used. Ten samples were collected in Summer (June and July) 2006 and sixteen were collected in Winter (December and January) 2006-2007. The specimens were fixed in 10% formalin, solution then the routine histological technique was performed. Three stains, Alum Hematoxylin and Eosin, Periodic Acid Schiff (PAS) Reagents and Van Giesons stain were used in this study. Slides then examined under the light microscope. The data were estimated by microoculometer (28 and 29). Analysis of data was done by using the analysis of variance (ANOVA) and T-test, least significant differences (LSD) were found between the mean of the data in Winter and Summer time at level 0.05% and 0.01% (30).

## RESULTS

### Thyroid gland:

The thyroid glands of the buffalo showed the common mammalian pattern of histomorphology. The capsule of the thyroid gland consists of two layers, the external one consists of loose collagen fibers interposed with large amount of adipose tissue, with few amount of elastic fibers. The inner one consists of bundles of collagenous and elastic fibers with few muscular cells. The capsule sends trabeculae which divides the gland into poorly defined lobules. The cellular boundries of the adipose tissue were more clear in Summer than in Winter time (Fig. 1, 2). The thyroid gland consists of small, medium and large sizes follicles. There were no significant differences between the follicular diameter in both seasons, moreover, there is no significant differences in the number of follicles in two seasons except in the small caudal follicles. The follicles have varying shapes (spherical, oval, tubular, polygonal and irregular). The small size predominate follicles were diffused at the periphery while the large follicles were present toward the centre (Fig. 3, 4 and Table 1, 2).

We can recognize two types of cells depending on the physiological status, viz., the first type has dark cytoplasm with pale blue spherical nuclei and vesicular appearance. While the second type has dark cytoplasm with dark purplish ovoid nuclei. The diameter of the follicular cells in both season showed no significant differences while the nuclei showed significant differences whereas, both cells and nuclei of isthmus showed significant differences (Table 3). One of the most striking result was the presence of obvious dark flat elliptical nuclei which belong to the contractile myoepithelial cells (basket cells) these cells lies peripherally to the follicles and

interposed between the follicular cells and its basement membrane (Fig. 5). Columnar follicular cells were not observed during both seasons. Small growing follicles were seen between follicles, some contains colloid, others were empty. The follicles of the isthmus were more regular than the other region of the thyroid gland.

In Summer season, the colloid was more homogenous and the periphery has as smoother profile than the Winter. Shrinkage due to fixation may be pronounced. The follicles were distended with colloid during both seasons. Little vacuoles may present at the free surface of the follicular cells. No remarkable differences between Summer and Winter in respect to the histomorphological picture of buffalo's thyroid gland.

The parafollicular cells have two locations throughout the parenchyma of thyroid gland i: e between follicles (parafollicular) which scattered singly, and interfollicular. There were significant differences in the diameter of parafollicular (C-cells) and their nuclei between two seasons (Table 3).

#### Accessory thyroid gland:

The epithelial lining of the accessory thyroid gland was low squamous to simple cuboidal. These cells were undifferentiated as it contained little amount of cytoplasm and dark ovoid nuclei. The follicles were predominantly inactive with large amount of interfollicular connective tissue (Fig. 6).

#### Parathyroid gland:

Our result revealed the presence of two pairs of parathyroid glands, external and internal. The external parathyroid gland lied adjacent to the thyroid gland at its cranial pole. A band of connective tissue capsule separate the two. The external parathyroid were surrounded by a well developed C. T capsule. It differs in structure from the internal one by the presence of a thick trabeculae which forms incomplete lobulation. The diameter of the external parathyroid was measured ( $9 \times 2.7$ )  $\mu\text{m}$ . The internal parathyroids may or may not be surrounded by a capsule. Its diameter ranges from  $2.25 \times 1.37$   $\mu\text{m}$ . The light chief cells of parathyroid occupy the centre of the gland. The gland consists of many types of cells lies in clusters, strands and sometimes it forms follicular arrangement. Four types of cells were recognized, there were large amount of the principle light and dark chief cells, syncytial and water-clear cells (Fig. 7, 8). Oxyphilic cells were not observed in the parathyroid gland of buffalo. The arrangement of parathyroid cells was more clear in Summer season (Fig. 7) in comparison with Winter (Fig. 9), moreover the nuclear and cellular limits were more prominent in Summer than in Winter.

**Table (1): The mean of different follicular number during different season of the thyroid gland (n=10 glands).**

Number of follicles/mm <sup>2</sup> in different season The part of gland	Summer			Winter		
	large mean $\pm$ SE	medium mean $\pm$ SE	small mean $\pm$ SE	large mean $\pm$ SE	medium mean $\pm$ SE	small mean $\pm$ SE
Caudal	7.7 $\pm$ 1.89 d	42.7 $\pm$ 1.36 b	50.03 $\pm$ 1.25 b	3.35 $\pm$ 0.37 d	42.0 $\pm$ 0.82 c	59.2 $\pm$ 3.89 a
Middle	5.5 $\pm$ 0.24 b	38.88 $\pm$ 2.03 a	55.63 $\pm$ 2.06 a	4.60 $\pm$ 0.15 b	50.02 $\pm$ 3.03 a	47.21 $\pm$ 2.43 a
Cranial	6.96 $\pm$ 1.69 a	39.63 $\pm$ 3.32 a	54.03 $\pm$ 2.87 a	4.26 $\pm$ 0.46 a	34.29 $\pm$ 3.32 a	61.47 $\pm$ 3.24 a
Isthmus	7.76 $\pm$ 1.45 a	58.76 $\pm$ 0.04 a	29.48 $\pm$ 4.26 a	4.09 $\pm$ 1.20 a	58.91 $\pm$ 0.05 a	28.19 $\pm$ 5.21 a

The number represent the mean  $\pm$  SE.

The similar letters represent no significant differences.

The difference letters represent significant difference at level of  $P < 0.05$ .

**Table (2): The mean diameter of cell and nucleus in different seasons (n=10 glands).**

Cell width and nucleus diameter and different season Part of gland	Cell		Nucleus	
	Summer	Winter	Summer	Winter
Isthmus diameter / mean $\pm$ SE	10.35 $\pm$ 0.195 $\mu$ m b	13.15 $\pm$ 0.34 $\mu$ m a	5.40 $\pm$ 0.121 $\mu$ m b	7.57 $\pm$ 0.196 $\mu$ m a
C cell diameter / mean $\pm$ SE	9.24 $\pm$ 0.14 $\mu$ m b	10.26 $\pm$ 0.15 $\mu$ m a	4.86 $\pm$ 0.13 $\mu$ m b	6.23 $\pm$ 0.12 $\mu$ m a
Follicular cell diameter / mean $\pm$ SE	9.94 $\pm$ 0.11 $\mu$ m a	10.02 $\pm$ 0.12 $\mu$ m a	6.41 $\pm$ 0.08 $\mu$ m b	7.04 $\pm$ 0.09 $\mu$ m a

The number represent the mean  $\pm$  SE.

The similar letters represent no significant differences.

The difference letters represent significant difference at level of  $P < 0.05$ .

**Table (3): The mean of different follicular diameter during different seasons in different part of thyroid gland (n=10 glands).**

The different cell in different season The part of gland	large		medium		small	
	Summer	Winter	Summer	Winter	Summer	Winter
Caudal diameter/mean $\pm$ SE	105.23 $\pm$ 1.84 a	94.92 $\pm$ 6.92 a	84.52 $\pm$ 9.9 0 a	69.61 $\pm$ 0.3 4 a	33.08 $\pm$ 2.2 6 a	38.0 $\pm$ 0.46 a
Middle diameter/mean $\pm$ SE	106.93 $\pm$ 12.4 6 a	124.02 $\pm$ 15.5 5 a	75.35 $\pm$ 1.0 9 a	75.45 $\pm$ 1.0 7 a	55.63 $\pm$ 2.2 6 a	47.21 $\pm$ 9.4 3 a
Cranial diameter/mean $\pm$ SE	118.77 $\pm$ 5.06 a	112.67 $\pm$ 2.16 5 a	73.78 $\pm$ 0.3 6 a	73.21 $\pm$ 2.5 5 a	30.73 $\pm$ 1.8 2 a	34.35 $\pm$ 0.0 3 a
Isthmus diameter/mean $\pm$ SE	102.25 $\pm$ 7.15 a	120.21 $\pm$ 6.46 a	74.33 $\pm$ 1.3 5 a	78.45 $\pm$ 2.2 9 a	29.71 $\pm$ 3.6 a	29.71 $\pm$ 0.0 6 a

The number represent the mean  $\pm$  SE.

The similar letters represent no significant differences.

The difference letters represent significant difference at level of  $P < 0.05$ .

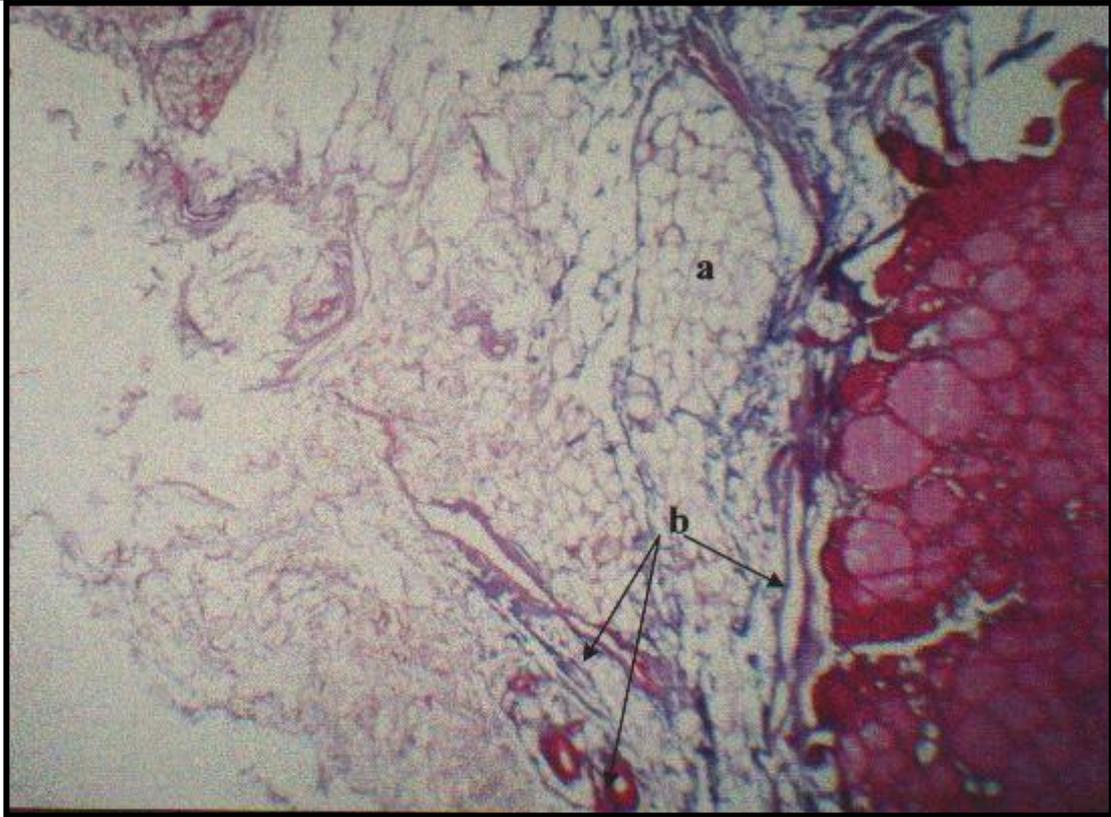


Fig.1: The capsule of thyroid gland (in Summer) a- adipose tissue b- blood vessels (arrows).PAS stain. X100.

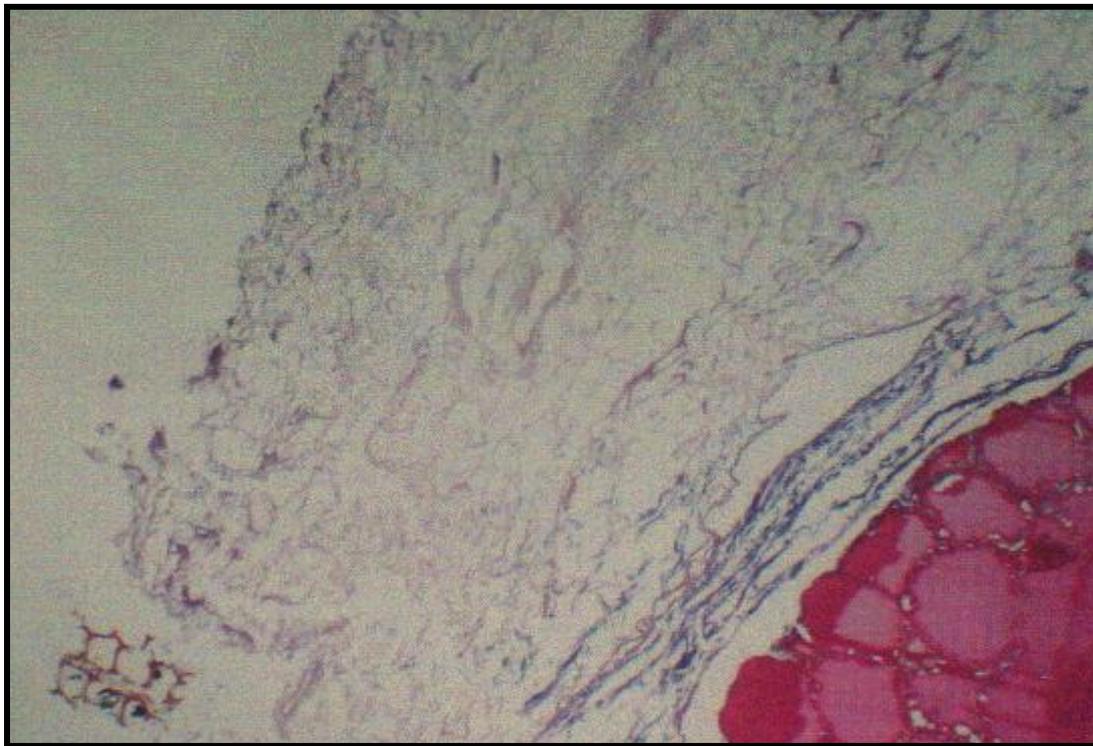
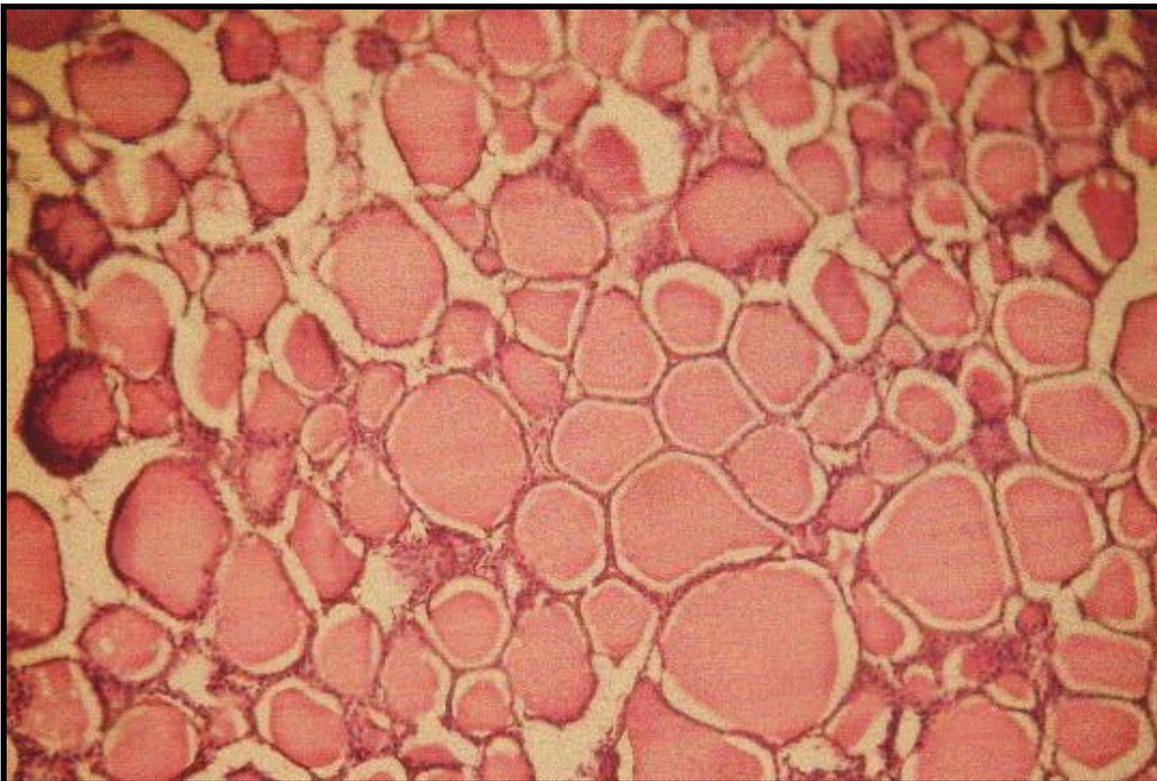


Fig.2: The capsule of thyroid gland (in Winter).PAS stain. X100.



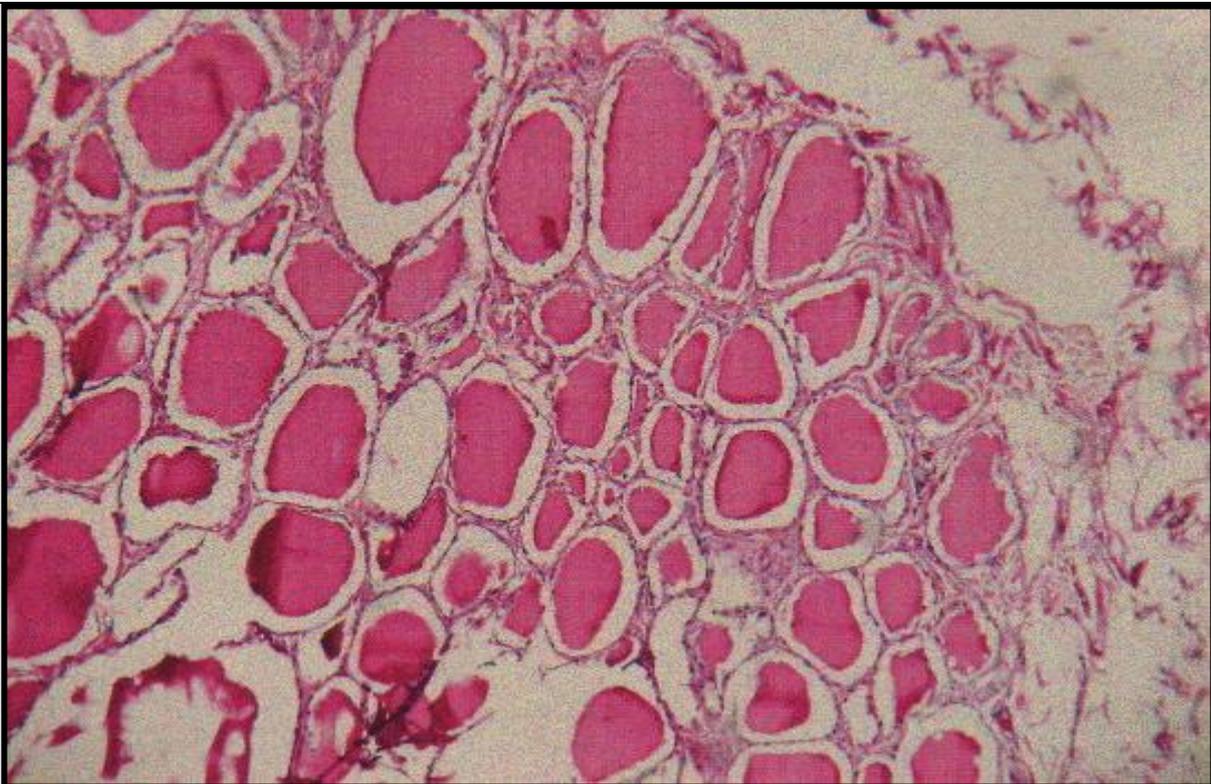
**Fig.3: Follicles of thyroid gland (in Summer).H & E stain. X100.**



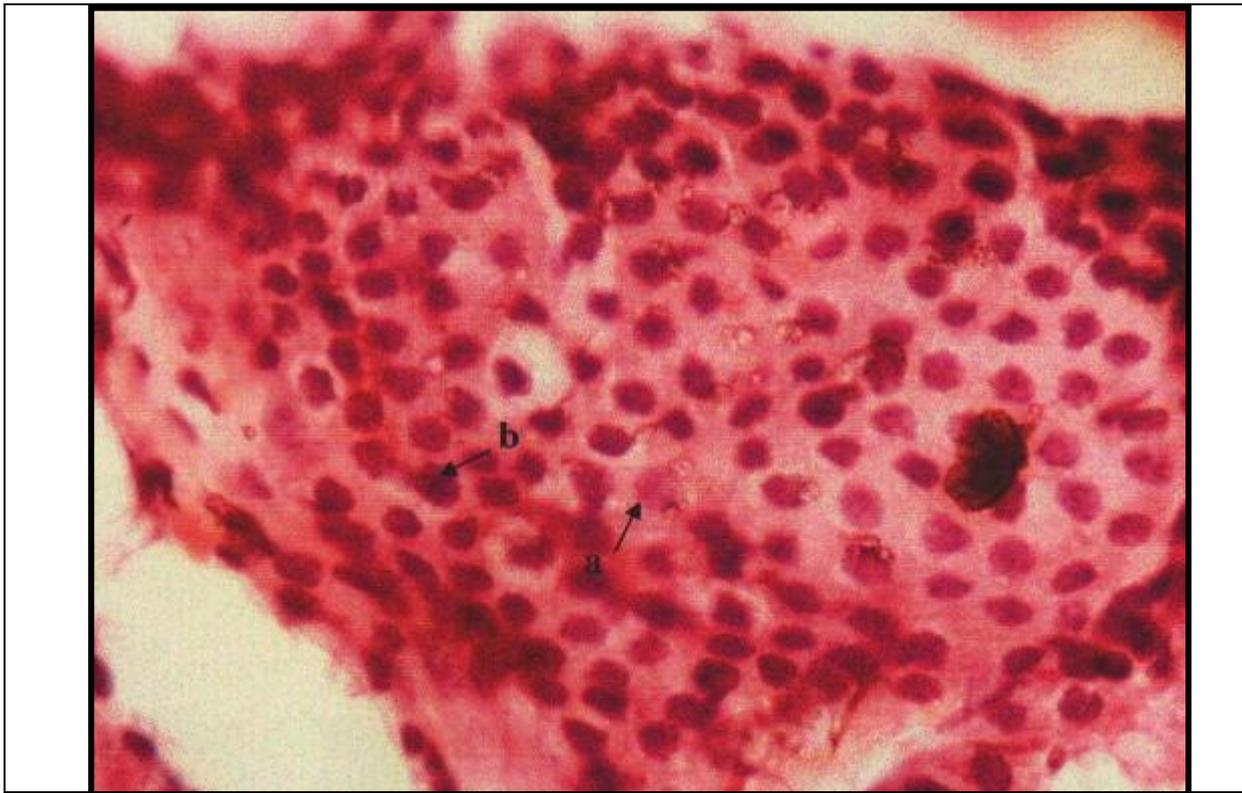
**Fig.4: Follicular of thyroid gland (in Winter).H & E stain. X100.**



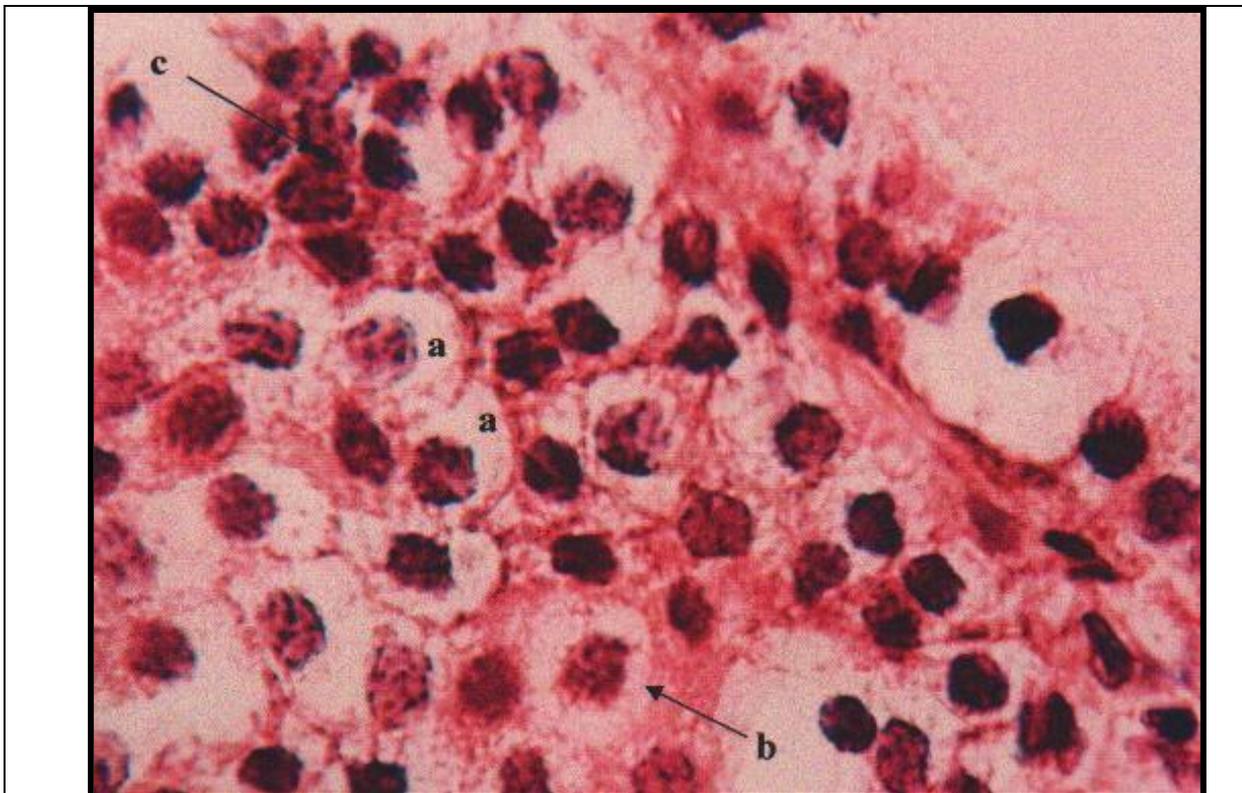
**Fig.5: Thyroid gland follicle a- myoepithelial cell b- follicular cell c- basement membrane d- fibroblast.H & E stain. X1000.**



**Fig.6: Accessory thyroid gland.H & E stain. X400.**



**Fig.7: Internal parathyroid gland (in Summer) a- light cell b- dark cell c- syncytial cells.H & E stain. X400.**



**Fig.8: External parathyroid gland a- water clear cell b- light chief cell c- syncytial cells.H & E stain. X1000.**



**Fig.9: Internal parathyroid gland (in Winter) a- fibroblast of the capsule b- light cells c- dark cell.H & E stain. X400**

## DISCUSSION

Our present findings referred to the presence of smooth muscle C-cells in the inner layer of the capsule, this may play a role in the contraction of the capsule to aid in the movement of thyroid colloid. This is in agreement with (31) in camel and (32) in sheep and goat. Our result regarded the capsule to consist of two layer, this is in contrast to (33) in buffalo who registered three layers, and invariance with (34) in Assam goat who regarded the thyroid capsule with a single layer. The presence of smooth muscle cells in the inner layer of the capsule, may play a role in the contraction of the capsule to aid in the movement of the colloid.

The thin trabeculae which present in the gland monitored to the low vascularization of each follicle, so that the thyroid gland of buffalo contains huge number of follicles and trabeculae to compensate this deficiency in blood nourishment. This is in agreement with (35) in the camel and (36) in buffalo who stated that the trabeculae were thin and do not form complete lobulation, moreover (31) recorded increase in thickness of the trabeculae of thyroid gland in Winter as this season regarded as the season of activity of thyroid gland in camel. It may be assumed that buffalo and cross breed cattle had higher heat load due to black hair and offered much less heat protection from sun than other animals, a question yet to be fully answered was why only the buffalo get this physiological behavior, the answer may be because buffaloes reserve a large amount of fat in its adipose tissue. Our herein result demonstrate the presence of growing follicles at the periphery and the developing larger ones at the centre. This is in agreement with (31 and 33) in camel who regarded that these growing follicles act as a nuclei for building new follicles in the parenchyma. He also regarded these growing follicles as an undifferentiated follicles which elicit proliferation and differentiation and rearrangement to become small new follicles. Our result was in contrast to (37) in goat who regarded this phenomena as due to the tangential sectioning and we regarded these growing follicles as a source to replace the large old follicles. These follicles grow in the

centre of the parenchyma and as they increase in size, they push the neighboring ones a way toward the periphery of the gland.

The dark nuclei of the follicular cells reflect that the nuclei was in resting state, this is in accordance with (12). The current study showed the presence of dark flat nuclei which lies around the follicles, the question raised was whether these nuclei were belong to the endothelium of the capillaries or to the connective tissue or myoepithelial cell nuclei. Our concept that these nuclei were belong to myoepithelial cells as its location was away from the capillaries endothelium and it was constricted between the follicular cells and the basement membrane (Fig. 5) to assist in contraction of the thyroid follicles then to move its contents. This is inforced by the presence of muscular cells in the inner layer of the thyroid capsule. This result in variance with (9) who state that these cells are especially well developed in sweat and mammary gland and in accordance with (38) who found the myoepithelial cells around the uniniferous tubules.

We suggest that parafollicular cells (C-cells) occupy only two locations, i: e interfollicular and parafollicular. It did not extend to the follicular lumen as till now it seems functionally that C-cells has no relation with the main function of thyroid gland. This is in contrast with (39) who added another location i: e intrafollicular. On the other hand the size of C-cells was larger in Winter than in Summer (Table 3). This means that the need of calcium was less in Winter. (5) Stated that the major role of calcitonin is to protect the skeleton against stress factors.

The follicular epithelium ranges between squamous to low cuboidal in both seasons. According to our survey of the literature, this finding has never been reported earlier in the thyroid gland of buffalo. Our concept that the buffalo was poor thermoregulator animal which have low simple sweat gland (40) and the thick dark discoloration of the skin and the presence of high storage of fat depot in its adipose tissue which act as a good insulator and a source of energy so that the buffalo need not another source of energy as the thyroid gland was regarded as a good source of energy due to its responsibility on metabolism through which, the body temperature elevated. The resting (inactive) thyroid gland leads to low basal metabolism which in turn exhibited better resistance to heat stress (41) as the increase basal metabolic rate will increase the oxygen consumption which produced heat (42). This will be serve as acclimatization and adaptative mechanism of the buffalo. Recent finding revealed that another organs in buffalo undergo adaptation to the harsh environment, this is assured by (38) who found that the buffalo has a large efficient kidney, which act to regulate body temperature, moreover (43) stated that the adrenal gland of buffalo play a double role in thermoregulation in both Summer and Winter.

#### **Accessory thyroid gland**

In light of our work there was a complete accordance with the finding of (44) who showed that the accessory thyroid gland was simple and contains predominantly inactive follicles with low epithelium. Our result declared that the endocrine organs usually related to accessory tissues or organs, this is mimics with the finding of (44)who found accessory adrenal gland in buffalo parathyroid gland.

#### **Parathyroid gland**

Many authors face difficulty in finding the external parathyroid gland owing to its small size and being embedded within the fat (45).

In this study, the external parathyroid was more developed than the internal one, it contains thick trabeculae which increase the level of vascularization and it seems that their cells were larger and more differentiated. Our recent work revealed that the parathyroid gland of buffalo was larger than that of horse (45). This means that this gland was more active than that of the horse. In all domestic mammals except sheep and goat, the dark and light cells of parathyroid gland were distributed randomly depending on the functional state of the gland. In sheep and goat the light chief cells occupied the periphery of the parenchyma, whereas the dark chief cells were in the centre. This is invariance with our result in buffaloes. As the parathyroid gland has one principle function which elaborates the hormone parathormone which regulate calcium level and bone

metabolism, so that, this gland need one type of cells to do this function. Our concept that the presence of many types of cells (light, dark chief cells, syncitial and water clear cells) may be a transitional stages which represent many functional variations of stem cells except oxyphilic cells which has acidophilic cytoplasm due to large amount of mitochondria (14) the latter cells were not observed in our study.

### دراسة نسيجية للغدة الدرقية والجنيب الدرقية في الجاموس العراقي

#### "*Bubalus bubalis*" مع الإشارة إلى التأثير الموسمي

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#### الخلاصة:

أشتملت الدراسة الحالية على جمع عينات الغدة الدرقية وجنيب الدرقية من مجازر وسط العراق في موسمي الصيف والشتاء لعامي 2006-2007. ثبتت العينات باستخدام الفورمالين 10% ثم اجريت عليها العمليات النسيجية الروتينية وصبغت بصبغات هيماتوكسلين-ايوسين، صبغة كاشف شف الدوري وصبغة فان كيزن. فحصت العينات تحت المجهر الضوئي باستخدام المقياس العيني المصغر لقياس الأبعاد للغدة النسيجية. ولغرض التحليل الإحصائي استخدم اختبار تي.

أهم النتائج هو عدم ظهور فروقات نسيجية خلال موسمي الصيف والشتاء في الجاموس وهذا مخالف لمعظم الحيوانات ولم يسجل من قبل الباحثين من قبل حيث أن الجاموس يعتبر حيوان ضعيف التنظيم التناضحي وأن الغدة الدرقية تشارك بالتنظيم الحراري فتقلل من نشاطها في كلا الموسمين للحفاظ على الحيوان بسبب احتواء جسمه على كميات كبيرة من الدهون تكون هي المسؤولة عن تزويده بالطاقة.

كانت الخلايا جنيب الدرقية (C-cells) قليلة ووجدت بصورة منفردة اضافة على ذلك تم تشخيص وجود أنوية الخلايا العضلية الظهارية حول الجريبات وهذا ما لم يذكره أحد من الباحثين من قبل. تم تشخيص وجود الغدة الدرقية الإضافية التي كانت ظهارتها ظهارة بسيطة واحتوت جريباتها على كميات قليلة من الغروان. كذلك اظهرت الدراسة وجود الغدتين جنيب الدرقية الداخلية والخارجية ولكن لم تلاحظ الدراسة وجود الخلايا الحمضة Oxyphils في الغدة جنيب الدرقية الداخلية.

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