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Thyroid activity of Emballonurid male bat, *Taphozous longimanus* (Hardwicke) during reproductive period

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Abstract

Thyroid gland in bat, *Taphozous longimanus* consists of two lateral lobes on the either side of trachea and are connected by isthmus. During the pre-breeding period large and medium sized follicles are predominant and small sized follicles are few while during the breeding period small and medium sized follicles are much more and large sized follicles are a few. Thyroid gland is composed of two different kinds of parenchymal cells, follicular epithelial cells and parafollicular cells ('C' cells or Calcitonin secreting cells or basal granular cell). 'A' type of follicular cells islined by tall columnar epithelial cells which are highly secretory in nature, 'B' type of follicular cells is lined by low columnar epithelial cells and are secretory in nature while 'C' type of follicular cells is lined by low cuboidal epithelial cells. Parafollicular cells during the breeding period are more in number and larger in size than the pre-breeding period. During the breeding period, weight of thyroid gland, the mean diameter of follicles, colloid and height of epithelial cells of small, medium and large sized follicles are more, but not significantly different from that of the pre-breeding period.

Keywords: Thyroid Gland, Histology, Bat, *Taphozous longimanus*, Follicles, Follicular Cells, Parafollicular cells, Light Microscopy

1 Introduction

The thyroid follicles are separated by a thin connective tissue stroma containing lymphatic vessel, blood vessels and nerves. The lumen of the thyroid follicle contains colloid, which is scalloped and pale in follicles with active secretory activity, densely eosinophilic in inactive follicles, and more flocculent ("like a clump or tuft of wool") and basophilic in the elderly man (Arrangoiz et. al.,

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2018). The second group of thyroid secretory cells is the parafollicular or C cells, derived from the neural crest, which contain and secrete the hormone calcitonin (Skandalakis, 2004).

Krishna and Singh (1998) studied the changes in thyroid gland with the reproductive cycle of *Scotophilus heathi*. Thyroid showed marked seasonal variation in weight, quantity of colloid and follicular epithelial height, suggesting the thyroid gland to be inactive during quiescence and winter dormancy and active during the time of recrudescence and breeding similarly to the testicular cycle. Plasma thyroxin (T4) concentration showed a significant seasonal change with high concentration during breeding and post-breeding and low concentration during quiescence. However, the T4 concentration increased from breeding to post-breeding phase, when the testes weight was declining. It is suggested that in S. heathi the positive correlation between thyroid and testicular cycles occurs only during the phases of the reproductive cycle when the body weight and testicular activity were also closely correlated.

Singh et al., (2002) investigated changes in thyroid activity during the reproductive cycle in the female bat, *Taphozous longimanus*. Thyroid gland showed marked seasonal variation in weight and secretory activity. The T3 and T4 concentrations were higher in recrudescence, late winter dormancy and minimum in quiescence and initial stages of first pregnancy. The correlation between body weight, thyroid weight and T3 and T4 concentrations in non-pregnant bats was higher when compared with pregnant bats. The T3 and T4 levels remained low during the initial stages of development in first pregnancy when compared with the initial stages of second pregnancy.

The thyroid gland of sexually immature dromedary camels (*Camelus dromedarius*) was studied using both light and electron microscopy (Abdel-Magied et al., 2000). The thyroid gland contained follicles of different sizes in both summer and winter. The large follicles were lined by very low cuboidal or semi-squamous follicular cells whereas the small ones were lined by high cuboidal or low columnar follicular cells. Electron microscopy showed that the very low cuboidal follicular cells were poor in organelles and were considered hypoactive. A few degenerated follicular cells were infrequently encountered in the camel thyroid. Parafollicular (C) cells were not seen in this study either with light or electron microscopy.

The detailed histological study of the thyroid gland of male *T. longimanus* during different period of the reproductive cycle has been undertaken because of its continuous reproductive behavior and to find out the probable role of thyroid gland in the control of reproduction. Therefore, the aim of the present study is to observe differentiation and activity of thyroid gland in *T. longimanus* during the reproductive cycle

2 Materials and Methods

Thyroid glands of *Taphozous longimanus* were fixed in the aqueous Bouin's for light microscopy. After 24 hours fixation, materials were washed in running tap water for 24 hours and dehydrated with upgraded series of ethyl, cleared in xylene and embedded in molten paraffin wax. Blocks were prepared, trimmed and cut into thin sections of 5-6 µ with the help of Leica 2417 microtome. The ribbon containing sections were spread and used for Hematoxylin-Eosin staining. For staining, slides were dewaxed in xylene, hydrated by downgraded series of ethyl alcohol upto the water, stained with Hematoxylin, washed in water and dehydrated by upgraded series of alcohol from 30% upto 70% ethyl alcohol. Slides were stained with eosin, washed in 90%, transferred in absolute alcohol, cleared in xylene and mount in DPX. The desired stained slides were observed under light microscope and micro-photographed at different magnifications.

3 Results

Observations

The thyroid gland is bilobed structure weighing 2-5 mg in *Taphozous longimanus*. It lies in the neck embracing the superior part of trachea and inferior part of cartilage and larynx. It consists of two lateral lobes on the either side of trachea and are connected by isthmus. The left thyroid lobe is triangular and right thyroid lobe is oval to spherical in shape (Fig.1 and Fig.2). Thyroid gland weight during the breeding period is significantly more than that of the pre-breeding period.The parenchyma of thyroid lobe contains many follicles. These follicles are varying in size and mostly spherical to oval or irregular in shape. Interfollicular stroma is mainly reticular and remarkably rich in vascular and capillary plexus. The histology of thyroid gland during different phases of reproductive cycle is as follows.

Thyroid Gland during the Pre-Breeding Period

The weight of left thyroid lobe is nearly equal to that of right thyroid lobe. It is composed of large no of follicles of different shapes and sizes. Each follicle is separated by Interfollicular connective tissue. For sake of describing the histological structure of different follicles in the thyroid gland, follicles are classified into three types on the basis of their diameter and epithelial height (Fig.1 and Fig.3).

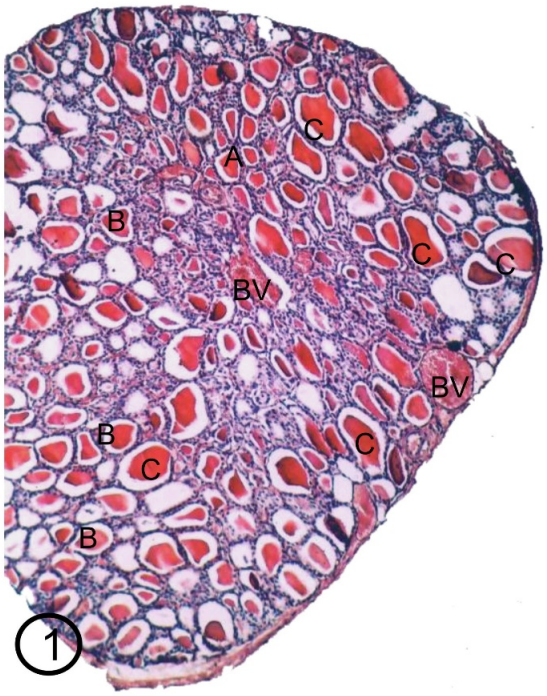

Figure 1. Light microscopic structure of transverse section of left thyroid gland during the pre-breeding condition consists of numerous peripherally arranged large follicles (C), medium follicles (B), few centrally arranged small follicles (A) and lumen consists of homogenous colloid (CO). Few blood vessels (BV) and capillaries are also observed in this section (WM). X 100

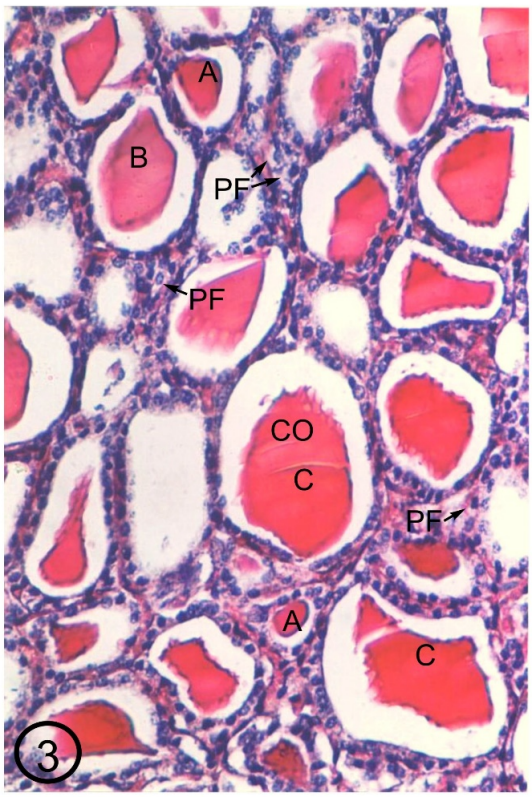

Figure 3. Transverse section of magnified part of left thyroid gland during the pre-breeding condition contains numerous large (C) and medium (B) follicles and few small (A) follicles. In the follicular lumen homogeneous colloid (CO) material are seen. The follicular cells of small follicles are low columnar, medium follicles are low cuboidal and large follicles are flat to rectangular in shape. Few large parafollicular (PF) cells are also noticed. X400

"A" type of follicles (Small sized follicles) - Diameter 20-40µ
"B" type of follicles (Medium sized follicles) - Diameter 41-70µ
"C" type of follicles (Large sized follicles) - Diameter 71-100 µ
The large sized follicles and medium sized follicles are predominant while small sized follicles are few during the pre-breeding period.

"A" type of follicles

These follicles are smaller in size and occur at the centre of the gland. These follicles are secretory in nature and bounded by loose connective tissue. The follicles are lined by low columnar epithelial cells with basophilic cytoplasm. The plasma membrane is not clearly visible. The nucleus is mostly spherical in shape and darkly stained. Nuclear membrane and chromatin clumps are clearly seen. The lumina are narrow and filled with basophilic colloid material. At the interphase of colloid and epithelial cells, few vacuoles are observed. Few microvilli are noticed, which projected into the lumen.

"B" type of follicles

These follicles are present at the middle region of thyroid gland. These follicles are larger than 'A' type of follicles. The number of these follicles is more than that of 'A' type of follicles. These follicles are less secretory in nature. Most of the follicles are lined by low cuboidal epithelial cells. The plasma membrane is not well defined. The nucleus is darkly stained and spherical to oval in shape. Chromatin material and nuclear membrane is clearly visible. Cytoplasm is less basophilic to more eosinophilic in nature. Homogenous bluish-purple colloid is observed in follicular lumina.

At the edge of colloid, numerous vacuoles are observed. Microvilli are clearly seen on apical region of follicular cells, which are projected into the lumen.

"C" type of follicles

These are the largest follicles during the pre-breeding period and predominantly occur at the periphery of thyroid. The number is more than 'B' type of follicles. These follicles are compactly arranged by thin connective tissue in thyroid lobe. Numerous follicles are incompletely filled with colloidal secretion and few follicles are empty. The follicles are lined by flat epithelial cells. The plasma membrane is not well differentiated. The nucleus is small, oval in shape and darkly stained. Nuclear membrane is clearly visible. Cytoplasm is scanty and eosinophilic in nature. Wide lumina of numerous follicles are filled with pink colored colloid, while in few follicles lumina are empty. Microvilli are not clearly seen.

Parafollicular Cells

These cells are found singly or in groups of 1-4 cells in the follicular epithelium and interfollicular connective tissue. The nucleus is eccentric, oval in shape and darkly stained. Nuclear membrane is clearly visible. Chromatin material is darkly and cytoplasm is faintly stained.

Thyroid Gland during the Breeding Period

The thyroid gland during this period is highly vascular. The weight of left thyroid lobe is nearly equal to that of right thyroid lobe. It composed of numerous follicles of different sizes. In this period smaller follicles and medium sized follicles are predominant while larger follicles are a few (Fig. 2 and 4). These follicles are widely dispersed throughout the cytoplasm and separated by interfollicular loose connective tissue.

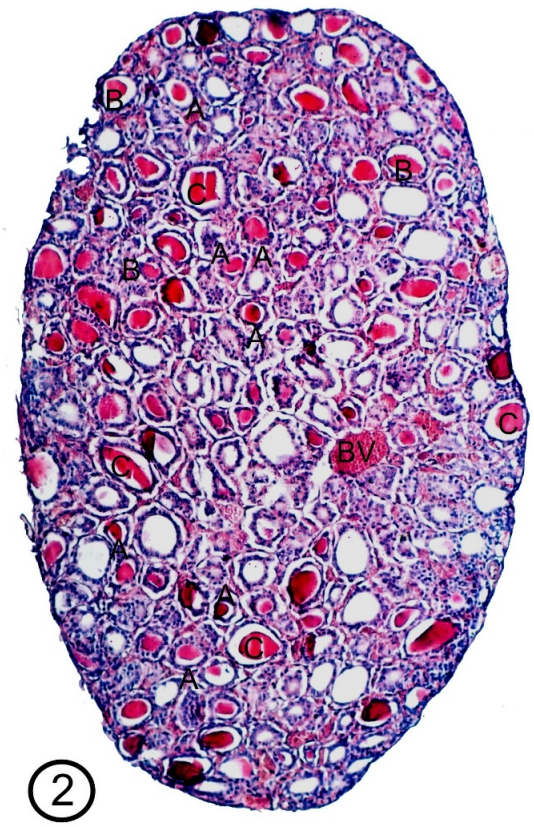

Figure 2. Light micrograph of transverse section of right thyroid gland during the breeding period consists of numerous small (A), medium follicles (B) and few large follicles (C). These

follicles are dispersed throughout the gland and contain homogenous colloid (CO) material in the lumen. Few blood vessels (BV) and capillaries are seen in the gland (WM). X100

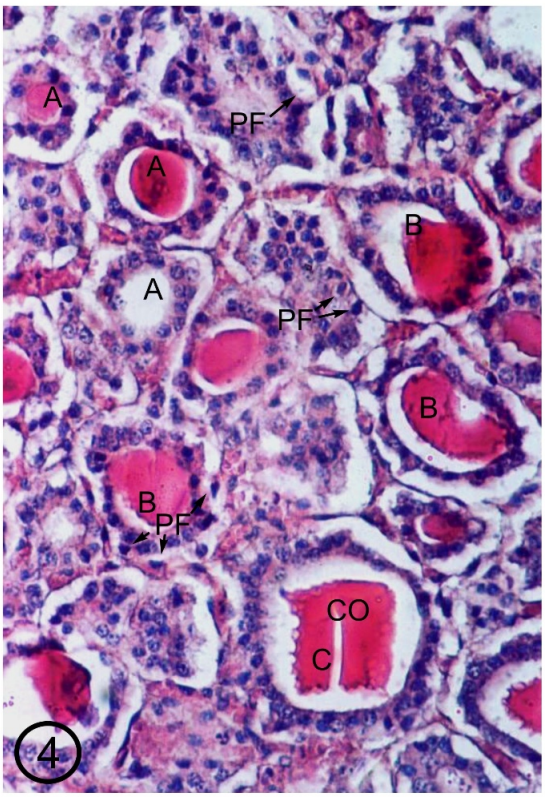

Figure 4. Transverse section of magnified part of right thyroid gland during the breeding period contains few large follicles (C), and numerous medium (B) and small follicles (A). The follicular cells of small follicles are tall columnar, medium follicles are low columnar and large follicles are low cuboidal in shape. Homogeneous colloid (CO) is seen in follicular lumen. Few large parafollicular (PF) cells are seen. X400

"A" type of follicles

These follicles are larger in size than the 'A' type of follicles during the pre-breeding condition and dispersed throughout the gland. The number of follicles is much more than the pre-breeding condition. These follicles are lined by tall columnar epithelial cells. The height of 'A' type of epithelial cells is more than that of 'B' and 'C' type follicular epithelial cells during the pre-breeding and the breeding period. These cells are highly secretory in nature. The nucleus is large spherical in shape and darkly stained. Chromatin material, nucleolus, and nuclear membrane are clearly visible. Cytoplasm is faintly stained and basophilic in nature. Plasma membrane is not clearly distinguished. Epithelial cell height is more than the pre-breeding condition. Numerous microvilli are projected into the lumen. Lumen is very small, which contained bluish colored colloidal material.

"B" type of follicles

These are medium sized follicles seen throughout the gland. These follicles are larger in size than 'B' type of follicles during the pre-breeding period. The number of these follicles is moderate but more than 'B' type of follicles during the pre-breeding period. These follicles are lined by low columnar epithelial cells and are highly secretory in nature. Plasma membrane is not clearly visible. Nucleus is large and spherical to oval in shape. The nuclear membrane is well developed with darkly stained

chromatin material. Nucleolus is eccentrically placed in nucleoplasm. Cytoplasm is basophilic - eosinophilic in nature. The microvilli are clearly seen towards lumen in some follicle. Lumina are filled with bluish-purple colored colloidal material.

"C" type of follicles

These follicles are large in size than 'C' type of follicles during the pre-breeding period. The number of these follicles is very less than the pre-breeding condition. These follicles are lined by low cuboidal epithelial cells. The plasma membrane is not clearly seen. Nucleus is small, mostly oval in shape and darkly stained. Chromatin material, nucleolus and nuclear membrane are clearly distinguished. Cytoplasm is scanty and lightly stained. Most of the lumina are empty while few are filled with colloidal material.

Parafollicular Cells

These cells are observed in follicular epithelium either singly or group of 3-4 cells in Interfollicular connective tissue. These cells do not reach to the lumen. These cells are more in number and larger in size than the pre-breeding period. Plasma membrane is clearly visible. Nucleus is oval or irregular in shape and darkly stained. Chromatin material and nuclear membrane are clearly visible. Cytoplasm is clear and lightly stained.

4 Discussion

The thyroid gland of *Taphozous longimanus* is composed of two different kinds of parenchymal cells, follicular epithelial cells and parafollicular cells ('C' cells or Calcitonin secreting cells or basal granular cell). The lumen is filled with colloidal material. Similar observations are reported in bat, *Taphozous kachhensis* (Bansod and Dhamani, 2014), *Myotis lucifugus lucifugus* as the thyroid follicle is made up of three principal components: the lining follicular cells, the luminal colloid, and the basal parafollicular cells (Nunez, et al., 1969; Kwiecinski et al., 1991) and camel (Ahmadpanahi, 2019).

In the breeding period, weight of thyroid gland, the mean diameter of follicles, colloid and height of epithelial cells of small, medium and large sized follicles are more, but not significantly different from that of the pre-breeding period. During the pre-breeding period large and medium sized follicles are predominant and small sized follicles are few while during the breeding period small and medium sized follicles are predominant and large sized follicles are few. In, bat, *Taphozous kachhensis*, thyroid glands during the sexually quiescence period, large and medium sized follicles are abundant and occupy middle or interior region of the gland while small sized follicles are less in number. While, during the sexually active period smaller and medium sized follicles are predominant while large sized follicles are few. These follicles are widely dispersed and separated by inter-follicular loose connective tissue (Bansod and Dhamani, 2014). In the thyroid gland of bat, *Scotophilus* heathi during November (Recrudescence) contains a rather homogeneous population of small to medium sized follicles lined by high cuboidal epithelium. Occasionally large follicles were also observed in the periphery of the gland. During breeding phase (February-March), thyroid follicles were generally larger than those observed in the winter dormancy. The epithelial cell height was also increased, but not significantly (Krishna and Singh, 1998).

On the basis of follicular size and epithelial height, the thyroid follicles of Mongoose, *Herpestes edawarsi* could be classified into three types. The small follicles were most active had a narrow lumen and tall columnar cells, the large follicles were characterized by low cuboidal cells and a large lumen and the medium follicles which were intermediate between the small and large follicles(Sapkal et al., 1977) supporting the present observations.

The small sized follicles in the thyroid gland during pre-breeding and breeding period of bat, *Taphozous longimanus* are lined by columnar epithelial cells and narrow lumen incompletely filled with bluish colored colloidal material. The nucleus is spherical, darkly stained, contains chromatin clumps. Few colloidal vesicles at the edge of colloid and few microvilli projected into the lumen are seen. The medium sized follicles consist of cuboidal to low columnar epithelial cells, spherical to oval shaped nucleus and bluish-purple colored colloidal material. The large sized follicles consist of low cuboidal epithelial cells, oval shaped nucleus and wide lumina contain pink colored colloid. In few follicles lumina are empty. Similar observations were reported in bat, *Taphozous kachhensis* (Bansod and Dhamani, 2014), *Scotophilus heathi* (Krishna and Singh, 1998), albino rat (Zaidi et al., 2004), *Herpestes edwarsi* (Sapkal et al., 1977), woodchuck (Krupp et al., 1976), Cream hamster (Neve and wollman, 1971), pig (Wagi, 1969) and camel (Ahmadpanahi, 2019).

The parafollicular cells during pre-breeding period occur singly or in group of 1-4 cells between follicular epithelium and interfollicular connective tissue but never reach to lumen. While in breeding period parafollicular cells are larger in size and more in number during the breeding period than the parafollicular cells during the pre-breeding period. The parafollicular cells are oval to irregular shaped, eccentrically placed nucleus contain chromatin material and covered by visible nuclear membrane. In thyroid gland of bat, *Taphozous kachhensis*, at the junctions of follicular cell represent the parafollicular cells. Inter-follicular stroma is mainly reticular and remarkably rich in vascular and capillary plexus. During the sexually quiescence period, parafollicular cells are found singly or in groups or 2-3 cells in the follicular epithelium and inter-follicular connective tissue. While in sexually active period parafollicular cells are observed in follicular epithelium singly or groups of 4-6 cells or in inter-follicular connective tissue (Bansod and Dhamani, 2014) which is similar with present Observations. In albino rat, the parafollicular cells are in the form of clumps having large round nuclei and eosinophilic cytoplasm (Zaidi et al., 2004).

The parafollicular cells are present between the follicular cells and basement membrane but never reach to the lumen. These cells are mainly single and roughly triangular to irregular in shape. Parafollicular cells are irregular, granular, and larger than the follicular cells, occur in group of 2-4 cells and are separated from the luminal colloid by the cytoplasm of the follicular cells in bat, Myotis lucifugus (Nunez et al., 1967, 1970) and in other mammals (Voitkevich, 1963 and Jubb et al., 1993). Parafollicular (C) cells are mainly single, occasionally two or more cells (Young and Lebland, 1963) and are observed between the follicular cells and basal lamina in close proximity to blood vessels in dog (Teitelbaum et al., 1970) and woodchuck (Frink et al., 1977) and camel (Ahmadpanahi, 2019). The above observations are in conformity with the present observations.

In bat, small follicles and tall columnar follicular cells have been associated with a high rate of activity(Nadler et al., 1954; Loewensteni and Wollman, 1967; Nunez, 1971). In the present observation, follicular cells and parafollicular cells are very active during breeding period than that of pre-breeding period. Therefore, it is states that thyroid glands of sexually active bat are directly or indirectly influencing the reproductive processes by metabolic effects in present bat, *Taphozous longimanus*.

Conflict of interests

There are no conflicts of interest.

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References

Abdel-Magied, E.M., Taha, A.A. & Abdalla, A.B. (2000). Light and electron microscopic study of the thyroid gland of the camel (*Camelus dromedarius*). Anatomia Histologia Embryologia, 29 (6), 331-336.

Ahmadpanahi, S.J. (2019). Presence of the Parafollicular Cells in the Thyroid Gland of the One-Humped Camel. Istambul University Cerrahpasa, Acta Vet Eurasia 2019.

Arrangoiz, R., Cordera, F, Caba, D., Muñoz, M., Moreno, E. & Luque de León E. (2018). Comprehensive Review of Thyroid Embryology, Anatomy, Histology, and Physiology for Surgeons. International Journal of Otolaryngology and Head & Neck Surgery, 7, 160-188.

Bansod D.S. & Dhamani A.A. (2014). Changes in the thyroid gland of the male emballonurid bat, Taphozous kacchensis (Dobson) during the reproductive cycle. Int. J. of Life Sciences, 2(3), 256-262.

Frink, R., Kropp, P.P. & Young, R.A. (1977). Seasonal variations in the morphology of thyroid parafollicular (C) cells in the woodchuck *Marmota monax*: a light and electron microscopic study. Anat. Rec., 189, 397-412.

Jubb, K.V.F., Kennedy, P.C. & Palmera, N.C. (1993). Pathology of Domestic Animals. Academic Press, Inc. Sam Diego, California. 4th ed. 2, 315-318.

Krishna, A. & Singh, K. (1998). Changes in the thyroid gland during the reproductive cycle of the male vespertilionid bat, *Scotophilus heathi*. Rev. Brasil. Biol., 58(4), 707-716.

Krupp, P.P., Young, R.A. & Frink, R. (1976) Thyroid gland of the woodchuck, Marmota monax: Morphological study of seasonal variations of the follicular cells. Anat. Record, 187, 495-514.

Kwiechinski, C.G. Damassa, D.A. & Gustafson, A.W. (1991). Patterns of plasma sex hormones binding globulin, thyroxine and thyroxine-binding globulin in relation to reproductive state and hibernation in female little brown bat. J. Endocrinol., 128(1),63-70.

Loewenstein, J.E. & Wollman, S.H. (1967). Diffusion of thyroglobulin in the lumen of rat thyroid follicle. Endocrinology, 81(5),1086-1090.

Nadler, N.J., Leblond, C.P. & Bogoroch, R. (1954) The rate of iodine metabolism by the thyroid follicle as a function of its size. Endocrinology, 54:154-172. Neve, P. & Wollman, S.H. (1971) Ultrastructure of the thyroid gland of the Cream Hamster. Anatomical record, 171, 81-98.

Nunez, E.A. (1971). Secretory processes in Follicular cells of the thyroid II. The occurrence of organelle associated intercellular junctions during late hibernation. Am. J. Anat., 131(2),227-240.

Nunez, E.A. & Gould, R.P., Hamilton, D.W., Hayward, J.S. and Holt, S.J. (1967). Seasonal changes in the fine structure of the basal granular cells of the bat thyroid. J. Cell. Sci., 2,401-410.

Nunez, E.A. and Gould, R.P. & Holt, S.J. (1969). A Study of granule formation in the bat parafollicular Cell. J. Cell. Sci., 5, 531-559.

Nunez, E.A., Gould, R.P. & Holt, S.J. (1970). Seasonal Changes in secretory granules and crystalloid inclusions of bat thyroid parafollicular cells. J. Cell. Sci., 6,821-842.

Sapkal, V.M., Baile, V.G. & Khamare, K.G. (1977). The histological and histochemical study of the thyroid gland of *Herpestes edwarsi* (Geoffroy). Journal of Shivaji University. 17, 103-111.

Singh, U.P., Krishna, A. & Bhatnagar, K. P. (2002). Seasonal changes in thyroid activity in the female sheath-tailed bat, *Taphozous longimanus* (Chiroptera: Emballonuridae). Acta Biologica Hungarica, 53(3), 267-278.

Skandalakis, J.E. (2004). Thyroid Gland. In: Skandalakis, J.E., Ed., Surgical Anatomy. The Embryologic and Anatomic Basis of Modern Surgery, Vol. 1, 14th Edition, Paschalidis Medical Publications, Athens.

Teitelbaum, S.L., Shieber, W. & Moore, K. E. (1970). C cell follicles in the dog thyroid: demonstration by in vivo perfusion. Anat Rec. 1, 69-77.

Voitkevich, A.A. (1963). Some main properties of parafollicular cells of thyroid gland. Arkli. Anat. Gistol. Embriol., 44, 52-56.

Wagi, B., (1969). Bulis: Nat. Inst. Anim. Ind. (Chiba), 19, 1-7.

Young, B.A. & Leblond, C.P. (1963). The light cell as compared to the follicular cell of the thyroid gland of the rat. Endocrinology, 73,669-686.

Zaidi T.M., Khan A.A, Hasan B.M. & Faruqi A.N. (2004) Carbimazole induced thyroid histopathy in albino rats during development. J. Anat. Soc. India, 53(2), 14-17.