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Original Article

Ultrastructural changes in parafollicular cells of Indian sheath-tailed bat, *Taphozous longimanus* (Hardwicke), during the reproductive cycle

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Summary

The Indian sheath-tailed bat, *Taphozous longimanus*, at Nagpur (Maharashtra State) shows continuous asychronous breeding, exhibiting aseasonal polyoestry. The morphology of parafollicular cells (C) cells in the thyroid gland of bat was studied during different phases of reproductive cycle. During estrus, parafollicular cells are elongated, irregular or oval and larger than follicular cells. Golgi apparatus is inconspicuous. Mitochondria, with lamellar cristae, are sparse. Rough endoplasmic reticulum occurs in the form of short tubular profiles dotted with ribosomes. Membrane-bound granules of varying size and electron density are observed in the cytoplasm. During pregnancy most of the parafollicular cells are packed with granules. The Golgi complex is inconspicuous and the rough endoplasmic reticulum is sparse. Multivesicular bodies are observed. During lactation, parafollicular cells exhibit morphological characteristics suggesting low synthetic activity and storage of granules. These morphological findings are discussed in relation to previous findings in other bats and with physiological activities of bat during the different stages of reproduction.

Key words : Bat, parafollicular cells, ultrastucture, reproductive cycle

Introduction

Thyroid gland of mammals is composed of two different kinds of parenchymal cells, parafollicular cells (C-cells or calcitonin secretory cells) and follicular epithelial cells. The C-cells in mammals are scattered throughout the follicular epithelium but are not in contact with the colloid. Nunez et al. (1967) found that thyroid gland in active bats, *Myotis* and *Nyctula*, has large number of granules-containing parafollicular cells which undergo alterations related to seasonal changes in the physiological state of the bat. Kwiecinski et al. (1987) reported that thyroid C-cells of *Myotis lucifugus lucifugus* exhibit maximum activity throughout the active period (summer). In *H. lankadiva* parafollicular cells are in active synthetic state during different phases of reproductive cycle (Seraphim, 2004). The thyroid parafollicular (C) cell has been shown to be source of calcitonin, a serum calcium-lowering hormone, and the locus of formation and storage of biogenic amines (Matsuzawa and Kurosumi, 1967; Ericson, 1972). Nunez et al. (1968) observed calcitonin, somatostatin and calcitonin gene-related peptide in parafollicular cells of bats.

Detailed study of the ultrastructural and functional characteristics of the parafollicular cells of *Taphozous longimanus* during different phases of the reproductive cycle has been undertaken because of its unusual reproductive behaviour, and to find the probable role of parafollicular cells in the regulation of physiological processes with special reference to reproduction in this species of bat.

Materials and Methods

Taphozous longimanus (Hardwicke) specimens were collected from Nagpur throughout the year, representing different reproductive states. The specimens were brought to the laboratory alive. Mature females were separated from immature females after observing mammary glands and pelvic dugs.

For histological examination, the thyroid glands from pregnant and non-pregnant bats were fixed in alcoholic Bouin's fluid. The tissues were dehydrated through graded series of ethanol, cleared in xylene, embedded in paraffin wax and sectioned at 5-6 ìm thickness in a Leica 2417 microtome (Leica, Jena, Germany). The sections were stained with Ehrlich's hematoxylin and eosin for microscopic observations (Pearse, 1968).

For electron microscopic studies, thyroid gland from pregnant and non-pregnant bats were fixed in fresh ice-cold 3% glutaraldehyde solution in cacodylate buffer for two hours. The tissues were washed in buffer and

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post-fixed for one to two hours in 1% osmium tetroxide. The tissues were dehydrated through graded series of alcohol, followed by propylene oxide, and embedded in araldite, which was be polymerized at 60^0C. Ultrathin sections were cut with glass knife, picked up on copper grids and stained with uranyl acetate and lead citrate. The grids were observed in a JEOL-100s Electron microscope (Japan) at 80KU accelerating voltage, and photographed.

Results

The parafollicular cells or C-cells of *Taphozous longimanus* show seasonal variation in their morphological characteristics. They are found in the interfollicular area of the thyroid gland. They are mostly seen as single cells but also found in groups of three to four cells. These cells are also included in the follicular wall but they never come in contact with the lumen.

The parafollicular cells during estrus are elongated or oval. They are electron-lucent and larger than the follicular cells. The nucleus is large and irregular in shape with indented margin and occupies a large portion of the cytoplasm. The nucleus contains finely condensed chromatin material, and chromatin clumps are seen along the nuclear envelope. Nuclear pores are seen. Rough-surfaced endoplasmic reticulum occurs as short tubular segments. Golgi apparatus is inconspicuous. Mitochondria are sparse and scattered throughout the cytoplasm. Some membrane-enclosed areas show a light flocculent material. They are of the same size as the dense granules or larger. Some dense granules are partly or totally degranulated. Small desmosomes and intercellular junctions are observed between the parafollicular cells and follicular cells (Fig.1).

During mid-pregnancy the parafollicular cells differ from those of the estrus thyroid, particularly in size and number of dense granules found in the cytoplasm. The nucleus is irregular in shape with clumps of chromatin in the nucleoplasm. The cytoplasm contains indistinct Golgi apparatus. The rough endoplasmic reticulum occurs in short segments, and a few free ribosomes are present. Mitochondria are rod-shaped, with lamellar or collapsed cristae, and are sparsely distributed. Membrane-bound granules of varying size and electron density are densely scattered in the cytoplasm. There are a few multivesicular bodies, containing about 3-4 vesicles in a matrix of moderate density, in the cytoplasm (Fig. 2).

During late pregnancy, parafollicular cell is filled with electron-dense granules of various sizes, shapes and electron densities. Mitochondria are rod-shaped, with lamellar cristae. The Golgi complex consists of dilated lamellae and small coated and non-coated vesicles. The cisternae of rough endoplasmic reticulum are in the form of short segments dotted with ribosomes. The large dense granules are partially or completely degranulated (Fig. 3).

During lactation, parafollicular cells are elongated or irregular and abut the interfollicular area. The nucleus is irregular and indented, and occupies a major portion of the cytoplasm. Chromatin material is fine and scattered throughout the nucleoplasm. Dense granules of varying size and electron density are seen at both the poles of the cell. Intercellular junctions between parafollicular cells and follicular cells are prominent. Other cell organelles are not conspicuous (Fig. 4).

Discussion

The 'C' cells sparsely populate the thyroid gland of *Taphozous longimanus* as in other mammals. However, they are quite abundant in the thyroids of hibernators (Azzali, 1967; Nunez, et al., 1967).

In *T. longimanus*, the parafollicular cells are present at the base of the follicular epithelium between the follicular cells. The cells are elongated, irregular or oval and are abetting to the interfollicular area as is the case in the bats *Myotis* and *Nyctula* (Nunez et al., 1967). The features such as Golgi apparatus being inconspicuous in some cells and poorly developed in others, sparse mitochondria with lamellar cristae, rough endoplasmic reticulum occurring in the form of short tubular profiles dotted with ribosome, membrane-bound round to oval granules of varying size and electron densities distributed throughout the cytoplasm, some of these granules appearing dense while others less electron dense, in some cells granules being either partly or totally degragulated, etc., are similar as in the bats studied earlier (Pearse, 1966; Nunez et al., 1967, 1969, 1970; Nunez and Gershon, 1972; Seraphim, 2004).

One of the major outcomes of this study is that the parafollicular cells in *T. longimanus* during estrus, pregnancy and lactation showed more or less similar ultrastructural features. The morphological characterstics of parafollicular cells in the thyroid gland of *T. longimanus* is suggestive of low synthetic activity and storage of granules during the reproductive cycle. This is different from the situation prevailing in *H. lankadiva* in which the parafollicular cells indicate features of high activity during the active phase. They have several dilated profiles of rough endoplasmic reticulum dotted with ribosomes. Mitochondria possess lamellar cristae. Several dense cytoplasmic granules of varying shapes and sizes are

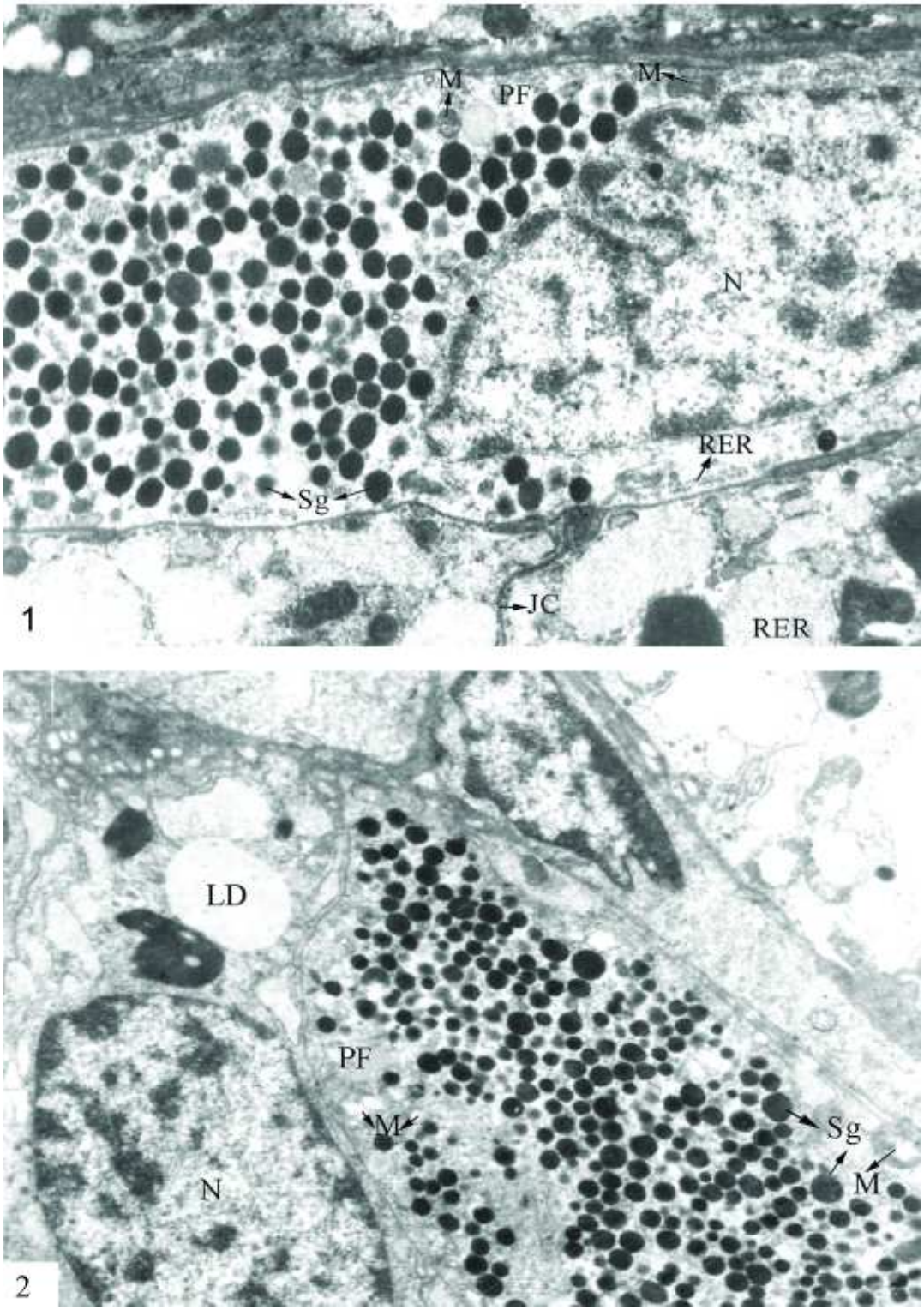
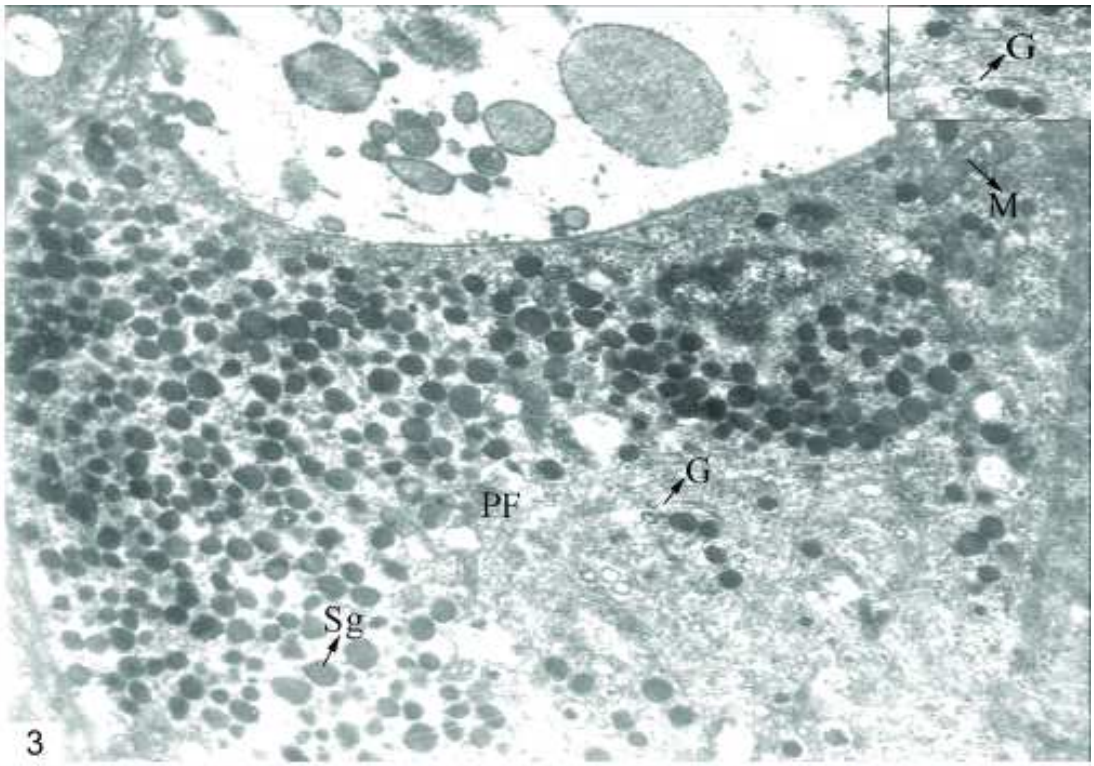


Fig. 1. Electron micrograph of parafollicular cell during estrus. Note large number of secretory granules (Sg) of varying size and electron density, one or two mitochondria (M), rough endoplasmic reticulum (RER) in the form of short tubular cisternae dotted with ribosomes and junctional complex (JC) between parafollicular cell and follicular cells. x 8000.

Fig. 2. Electron micrograph of parafollicular cell during mid-pregnancy. Note several secretory granules (Sg) of different shapes and sizes and electron densities in the cytoplasm. Electron opaque lipid droplet (LD) is seen in the follicular cell. x 8000.



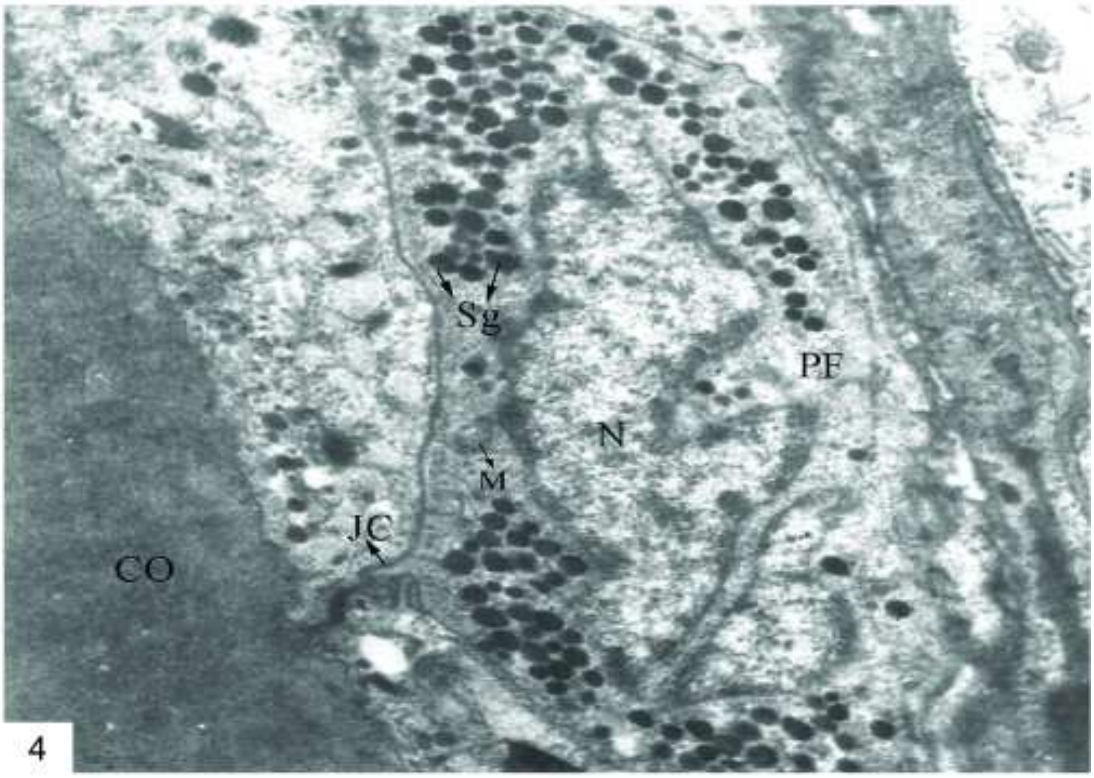


Fig. 3. Electron micrograph of parafollicular cell during late pregnancy, showing large number of secretory granules (Sg) of varying sizes and electron densities. Golgi apparatus (G) is distinct. Mitochondria (M) are rod-shaped. x 8000.

Fig. 4. Electron micrograph of parafollicular cell during lactation. Note indented nucleus (N), secretory granules (Sg) of various sizes and electron densities and intercellular junction (JC) between parafollicular cell and follicular cell. x 8000.

observed in the cytoplasm. All these features suggest that the cell is in active synthetic state during the different phases of the reproductive cycle (Seraphim, 2004). In the active *Myotis* and *Nyctula* the prominent granular endoplasmic reticulum consists of long tubular channels which are mostly arranged in parallel stacks. Clusters of free ribosomes are noticed in the cytoplasm (Nunez et al., 1967).

Nunez et al. (1967) studied the cycle of intracellular changes in the parafollicular cells and found that thyroid gland in active bats, *Myotis* and *Nyctula*, have large number of granule-containing parafollicular cells which undergo alterations in relation to seasonal changes in the physiological state of the bat. Some of the parafollicular cells of bats caught in late summer contain small dense secretory granules packed in the Golgi apparatus, while the large granules are found enclosed within a ribosome-studded membrane which may increase by direct accretion of newly synthesized material at their surface (Nunez et al., 1969). During early hibernation, the large dense granules are absent, while cells containing small granules are either partly or totally degranulated. Similar observations have been reported for parafollicular cells of estrus and pregnant bats of *T. longimanus*. Nunez et al. (1967, 1969, 1970) suggest that the two types of granules present in bat thyroid parafollicular cells have different fates during the annual cycle of physiological activity. In contrast to parafollicular cells of hibernating bats, the parafollicular cells of *T. longimanus*, a non-hibernator, contain a single type of granules.

The C-cells of *Myotis lucifugus lucifugus* exhibit maximum activity through out the summer active period. This may be correlated with maintenance of plasma calcium concentration during lactation, when female skeleton undergoes a period of bone demineralization (Kwiecinski et al., 1987). However, the parafollicular cells of *T. longimanus* show storage of secretory granules during different phases of reproductive cycle.

Biochemical and ultrastructural studies strongly suggest that the small granules are intracellular storage sites of calcitonin (Pearse, 1966). The number of these granules decreases greatly following experimental elevation of blood calcium. The change in form and number of small granules in parafollicular cells during hibernation reflects the release of calcitonin in response to the transient high blood calcium level which develops in hibernating bats. However, this rise in blood calcium is followed by a hypocalcemic state during extended hibernation (Azzali, 1968).

There are two opinions regarding the role of parafollicular cells in respect of the large granules. First, these granules may be a special form of the smaller kind and so also store calcitonin (Pearse, 1966; Bussolati and Pearse, 1967; Ericson, 1972). Second, the large granules may be storage sites for some quite different cell products such as biogenic amines, e.g., serotonin (Larson et al., 1966). Serotonin is present in some parafollicular cells in active bat (Gershon and Nunez, 1970; Nunez and Gershon, 1972) and rat thyroid (Matsuzawa and Kurosami, 1967). This biogenic amine has been implicated in the regulation of thyroid cell activity (Haymovites et al., 1976). The thyroid gland activity, which accompanies hibernation in some species, may also occur in the bat and be partly controlled by the local action of serotonin release from the large granules. Therefore, we conceive that production of granules in parafollicular cells during estrus, pregnancy and lactation in *T. longimanous* does have a purpose and suggest that calcitonin has a physiological role throughout the reproductive period. Thus, homeostasis would be maintained through fine regulation of the balance between blood and bone calcium during the reproductive period.

It has been suggested that the parafollicular cells of bat secrete calcitonin and somatostatin (Nunez et al., 1968). Calcitonin normally acts on bone to prevent calcium release. Secretion of the hormone by bat parafollicular cells may slow down bone resportion during hibernation. When animals are quiescent for a long period there would be lack of stimulation by mechanical stress which would in turn lead to bone resorption and hence to an elevation of the serum calcium level (Whalen et al., 1971). Interestingly, *T. longimanous* does not hibernate. It is a continuous breeder. We suggest that the parafollicular cells in this bat are in a reserve state during different phases of the reproductive cycle as indicated by ultrastructural characteristics. It is likely that they are responding to or perhaps controlling the seasonal changes in the plasma calcium concentration.

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