THYROID AND REPRODUCTION IN THE BA T 707

CHANGES IN THE THYROID GLAND DURING THE REPRODUCTIVE CYCLE OF THE MALE VESPERTILIONID BAT, Scotophilus heathi

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ABSTRACT
The aim of present study was to compare the changes in thyroid gland with the reproductive cycle of S. 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 are also closely correlated.

Key words: Thyroid, thyroxin, testes, bat, body weight, reproduction.

INTRODUCTION
Temperate-zone vespertilionid bats exhibit several unique features in their reproductive cycle such as prolonged sperm retention in the epididymis, asynchronous renewal of gametogenesis and the accessory reproductive organs and asynchrony of the period of spermatogenesis and copulation (Gustafson, 1979, 1987). It is suggested that these peculiarities associated with reproduction in the temperate-zone vespertilionids have evolved as a result of the superimposition of the period of hibernation on the season of reproduction (Racey, 1982). Even though, S. heathi, a subtropical...
vespertilionid, does not undergo prolonged hibernation, these bats exhibit peculiarities in reproductive process as demonstrated in temperate-zone vespertilionid bats. Previous studies from our laboratory have showed an increase in the circulating androstenedione concentration during the period of October to December, which was higher than androstenedione concentration reported in most mammalian species (Singh & Krishna, 1995, 1996). This increase in unusually high circulating androstenedione concentration may be responsible for the unique reproductive features noticed in *S. heathi*. A direct correlation between the increase in body weight and circulating androstenedione level increases during the period of weight gain, and declines during the period of weight loss. These studies suggested that nutritional changes can influence gonadal steroidogenesis in the species. It is therefore possible that the reproductive activity in *S. heathi* may be influenced by changing metabolic status.

The metabolic hormone, thyroxine (T₄), has been implicated in the physiological regulation of energy balance as well as in maintaining normal reproductive function in mammals (Schwartz et al., 1992; Boswell et al., 1994; Shi & Barrell, 1992). Further it has been reported that a reduction in the secretion and plasma levels of gonadotrophins was associated with hypothyroidism (Hagino, 1971; LaRochelle & Freeman, 1974; Buchanan et al., 1977). Likewise, Dunn et al. (1976) have shown that the daily rhythmic release of luteinizing hormone (LH) and prolactin in rats was altered by thyroidectomy. Subsequently, thyroid hormones were shown to be required for a normal transition from the reproductively active to inactive condition and vice versa in several seasonally breeding avian and mammalian species (Shi & Barrell, 1992). Although there are some studies on the thyroid gland of bats (Hudson & Wang, 1979) it would be interesting to find out the relationship between thyroid and testes in the bat showing unique reproductive features related to metabolic changes. Therefore, the pattern of seasonal changes in the thyroid gland of *S. heathi* was studied in relation to the changes in its reproductive cycle.

**MATERIALS AND METHODS**

All bats were trapped alive in the areas adjacent to Banaras Hindu University Campus. Body weight of each bat was recorded. Based on the reproductive cycle of *S. heathi* (Krishna & Singh, 1997), males were classified into the following five stages:

1. **Quiescence (May to August)**: Testis do not show spermatogenesis, the accessory sex glands are regressed and there is no sperm in epididymis.

2. **Recrudescence (September to early November)**: Testis showing spermatogenesis, the accessory sex glands are non-secretory but sperm is present in epididymis.

3. **Breeding (Mid-November to early December; February to March)**: Testis showing spermatogenesis, the accessory sex glands are active and sperm is present in epididymis.

4. **Winter dormancy (Mid-December to Mid January)**: Testis not showing spermatogenesis, the accessory sex glands are regressed but sperm is present in epididymis.

5. **End of breeding (Late March to April)**: Testis showing regressive changes, the accessory sex glands are regressed but sperm is present in epididymis.

**Collection of Serum and Tissues Histology**

The male bats were sacrificed as soon as they arrived in the laboratory. Their blood serum was collected and stored in –20°C until assayed. Testis and thyroid were excised out from the body cavity and excess fat and connective tissue attached were separated out. All the tissues were fixed in Bouin’s fluid for 24 hr., followed by preservation in 70% ethyl alcohol. Each tissue was weighed separately after placement in 70% alcohol. The tissues were dehydrated in ethanol, cleared in oxylol, embedded in paraffin wax, serial sections at 6 µm were cut and stained with haematoxylin and eosin.

**Morphometry**

Thyroid follicular epithelial height was measured using optical micrometer. Measurements were taken from at least 10 different randomly selected thyroid follicles from each bat. The areal fraction of the colloid within a particu-
lar section of the thyroid gland was estimated using standard point counting techniques (Weibel, 1979; Singh & Krishna, 1996). Three to five sections regularly spaced throughout the thyroid of each bat were examined at 100 x magnification under a microphat FX microscope (Nikon) attached to a TV monitor fitted with a square lattice containing 560 crosses. The sections used for morphometric analysis were selected by systematic random scheme (West, 1993). The first section was selected from the first five serial sections of the thyroid, and then every 20th serial section was examined. The number of crosses falling on the colloid or epithelial cells (hits) and over the entire thyroid section were counted. At 140X magnification, these crosses fell 70 µm apart. The number of points falling on the colloid (P) and over the entire thyroid section (Pt) was obtained for each section examined. The areal fraction ($A_a$) was then calculated using the formula: $A_a = (P_i)/(Pt_i)$, where $P_i =$ number of hits in section i, $Pt_i =$ number of test point in section i. The mean areal fraction was then calculated from the data ($A_a$) obtained from each thyroid examined per group. The thyroid from at least 4 males from group were examined for morphometric analysis.

Hormony Assay

Thyroxine: The circulating T4 concentrations was measured by radioimmunoassay using RIAK-5 kit obtained from Bhabha Atomic Research Center, Bombay, India. Serum sample (10 µl) in duplicate was used for assay. Four different T4 standards (2.5, 5, 10 and 20 ng/ml) were used. To all samples and standards in 100 µl of assay buffer was added 100 µl each of 125I-T4 and T4 antiserum. After mixing the tubes gently, all the tubes were incubated at 37°C for 30 mins. One ml of polyethylene glycol solution was added to all tubes except total count, mixed well and centrifuged at 2000 xg for 20 mins. Supernatant was discarded without disturbing the precipitate. Average counts per minute (cpm) was taken for all the duplicate tubes in a Beckman Gamma Counter.

Statistical analysis

The data were analysed by one-way analysis of variance followed by Duncan’s test and correlation coefficient were also applied. Differences were considered significant if $P < 0.05$. Data are expressed as mean ± S.E.

RESULTS

Seasonal changes in body, Testis, and Thyroid Weights

The weights (mean ± SE) of the body, testis, and thyroid during reproductive phases are shown in Fig. 2. There was more than two fold difference between the highest and lowest mean thyroid weight of adult bats caught during different reproductive phases. The thyroid weight showed two peaks coinciding with the peaks of testicular weight during recrudescence and breeding phases. Both thyroid and testes began to increase in weight from quiescence (August) and attained a peak during recrudescence (November). Thyroid weights declined during winter dormancy similarly to testis weight.

Histology

Thyroid gland of S. heathi also showed marked seasonal variation in histology (Fig.1). The percent areal fraction of colloid in the thyroid gland showed a seasonal variation, with higher values after the end of breeding and lower values during the recrudescence (Fig. 3). Thyroid follicular epithelial cells height also showed a significant increase during the recrudescence stage (Fig. 3).

During quiescence in August, the thyroid was extremely heterogeneous in appearance showing great variability in the height of the epithelium and in the amount of colloid in the various thyroid follicles. The cells of majority of follicles during this phase contain flattened cells having scanty cytoplasm and a small nucleus. Some of the thyroid follicles were empty and others contain only small amounts of colloid in their big lumen. Numerous small follicles containing little colloid were found in the central part of the thyroid. These follicles are lined with cuboidal epithelial cells.

In contrast to the heterogeneous appearance during the quiescence phase, the thyroid during November contain a rather homogeneous population of small to medium sized follicles. These follicles were lined with high cuboidal epithi-
Occasionally large follicles were also observed in the periphery of the gland.

The thyroid gland during winter dormancy showed mostly smaller follicles, which were lined with low cuboidal epithelium but containing large and prominent nuclei (Fig. 1a). Large follicles located in the periphery of the gland filled with colloid were lined with squamous epithelium. The epithelium of the winter thyroid is generally smaller than that of the recrudescence animals and because of this the glands tend to have a less cellular appearance.

During breeding phase in February-March, the thyroid gland contains follicles which were generally larger than those observed in the winter dormancy (Fig. 1b). The epithelial cell height was also increased, but not significantly. During the post-breeding phases, in April-May, the thyroid gland contained mostly medium sized follicles with large lumen completely filled with colloid and low cuboidal epithelial lining (Fig. 1c).

Serum thyroxine concentration

Serum T₄ concentration showed a significant (P < 0.05) seasonal pattern of changes (Fig. 3) with the highest concentration observed during April-May (Post-breeding phase) and lowest in August (quiescence). Concentration of T₄ increased significantly during November (recrudescence) as compared to August (quiescence). T₄ concentrations remain statistically unchanged from November until March (breeding).

DISCUSSION

Thyroid is a metabolically important gland, which is suggested to be essential for the normal maintenance of reproductive function, impairment of thyroid activity may be inhibitory to reproduction (Peebles et al., 1984; Jannini et al., 1995). The present study also showed a close relationship between changes in the weight and morphological features of the thyroid and the testicular cycle of S. heathi. The thyroid weight was found to be low during quiescence and winter dormancy and increase during recrudescence and breeding phases similar to the changes in the testicular weight, although a significant increase was noticed only during breeding but not during recrudescence (Fig. 1). The increase in thyroid and testes weight from quiescence to recrudescence and the decrease from recrudescence to winter dormancy were also found to be similar to the pattern of changes shown in the body weight in this species. This indicate that changes in the body, thyroid and testes weights in this species are closely correlated during the period from quiescence to winter dormancy. A significant correlation between body weight and testis has recently been demonstrated during breeding cycle in this species (Singh & Krishna, 1996). In S. heathi the body weight increases due to fat accumulation during the period of increased food intake and decreases due to metabolism of stored fat during of food scarcity (Singh & Krishna, 1996). This suggests that the increase in both thyroid and testes weight from quiescence to recrudescence and decrease from recrudescence to winter dormancy may be related to seasonal body fat or nutritional cycle. Further changes in the thyroid and testicular activity in this species differ markedly from breeding to post-breeding phase. Thyroid weight and T₄ secretion increased whereas the testis weight and spermatogenic activity declined significantly from breeding to post-breeding phase (Krishna and Singh, 1997).

However, the body weight did not change significantly during this period. This indicate that changes in the thyroid, testes and body weights are not inter-related with each other during the period from breeding to post-breeding. It thus appears that thyroid and testes in S. heathi are correlated when the body weight is significantly correlated with the testes weight. Whereas changes in thyroid and testes are not related with each other when the changes in the body weight has no correlation with testicular activity.

This suggest that either nutritional changes have affected both thyroid and testes via common hypothalamic-pituitary axis (Cosgrove et al., 1995) or nutritional changes have affected testes via thyroid. Further studies are required to confirm the relationship between body weight, thyroid and testes in this species.

Among bats, the thyroid gland was studied mainly in the temperate-zone bats that undergo prolonged hibernation (Sadler & Tyler, 1960a, b; Azzali, 1964; Velicky & Tiltbach, 1972, 1974; Burns et al., 1972; Ifuta et al., 1988). Thyroid function in these bats, as in other mammalian hibernators, exhibit marked seasonal fluctuations.
Fig 1 — All the figures are transverse section of the thyroid gland of male *S. heathi* during different stages of reproductive cycle. (a) Thyroid during winter dormancy (December) showing comparatively smaller follicles x75; (b) Thyroid during breeding (March) showing larger follicles as compared to winter dormancy x75; (c) Thyroid during post-breeding (April) showing mostly medium to smaller sized follicles x75.
In general, thyroid activity in these bats has been found to increase in the spring, decrease in autumn and remain quiescent throughout most of hibernation. Plasma T\textsubscript{4} in adult male bats, *Myotis lucifugus*, was found to exhibit a well-defined annual cycle. Levels were lowest at the time of entrance into hibernation in the fall, increased during hibernation, and attained a maximal values in the spring at the time of arousal (Damassa et al., 1995).

However, no such studies are carried out in any of the tropical/subtropical bats. Histology of thyroid in *S. heathi* also showed changes comparable to its weight cycle. Based on the seasonal changes in the quantity of colloid and in the height of the epithelial cells of the thyroid gland, it can be concluded that the gland appeared inactive during the winter coinciding with the suppression of spermatogenesis and most active during the time of breeding and post-breeding phases. In general, the seasonal cycle of serum T\textsubscript{4} concentration supported the morphometric finding on thyroid gland except during winter dormancy. In *S. heathi*, T\textsubscript{4} level was observed to be lower in summer just before the onset of reproduction and higher in spring during post-breeding phase.

The present study thus correspond well with the previous findings indicating that animals living free have inactive thyroids in the summer period. Lower circulating thyroxin levels in the summer are in good correlation with the lower thyroid activity. There was marked activation of structure and gain in weight of thyroid during the recrudescence phase when compared with quiescent bat. This may indicate that activation of thyroid gland in *S. heathi* coincided with the period of transition from the non-breeding to the breeding state and may be necessary for this transition as shown in other seasonally breeding animal (Webster et al., 1991).

*S. heathi* during winter dormancy exhibits unique condition regarding thyroidal activity. Similar to most hibernating mammals (Hoffman, 1964; Hudson & Wang, 1979), thyroid gland of *S. heathi* appeared inactive during the winter. However, circulating T\textsubscript{4} concentration during this period remained high. Thus, they resemble the chipmunk, *Tamias striatus*, in which the plasma T\textsubscript{4} level remains the same for hibernating or normothermic animals (Hudson, 1977). T\textsubscript{4} level was also shown to be high during hibernation in *Myotis lucifugus* (Damassa et al., 1995). The mechanism(s) responsible for the elevated serum T\textsubscript{4} levels in *S. heathi* during winter dormancy, a time of relative metabolic inactivity, are not clearly understood. It may be presumed that the increase in T\textsubscript{4} concentration may be due to enhanced secretion or to decreased metabolism and disposal or to both of these factors.

Observations of enhanced binding of T\textsubscript{4} by thyroid binding globulin (TBG) in hibernating ground squirrels and wood chuck have led to the suggestion that elevated TBG levels are partially responsible for winter associated increase in serum concentration of thyroid hormones (Magnus & Henderson, 1988; Young et al., 1986). The hibernating bat, *Myotis lucifugus* also showed high TBG levels during winter (Damassa et al., 1995). Further, decrease in weight and inactive appearance of thyroid during winter could be due to decline rate of thyroglobulin synthesis of regressed epithelial cells. But increase of T\textsubscript{4} could be due to release of the stored T\textsubscript{4}. It may be noted here that the gland contains a large extracellular store of its products which might suffice for prolonged secretion in the absence of synthesis. Another reason of high T\textsubscript{4} in the circulation could be due to decreased peripheral conversion of T\textsubscript{4} to T\textsubscript{3} because of a lowering in liver to T\textsubscript{4}-5-monodeiodinase (Webster et al., 1991; Decuypere et al., 1985). It has also been shown that during fasting plasma T\textsubscript{3} concentration decreases due to decreased conversion from T\textsubscript{4} (Cameron, 1996). *S. heathi* may require a normal T\textsubscript{3} to stimulate sufficient thermogenesis during torpor to prevent body temperature from falling below a critical level that would necessitate arousal. The proposed hypothesis may also explain why the bat do not drop their body temperature much below 20-24°C during winter dormancy (Hulbert, 1978). Ground squirrel, which have low T\textsubscript{4} during most of their hibernation periods, can let their body temperature drop to 0°C (Lyman & Chatfield, 1955).

None of the various endocrine glands thought to play a role in reproduction has been more thoroughly studied with more conflicting results than the thyroid gland. As far as gonadal-thyroid interrelation in *S. reathi* is concerned, two different patterns were noticed.
Fig. 2 — The body, testes, and thyroid weights of male *S. heathi* during different reproductive stages. QUI = Quiescence; REC = Recrudescence; WIN = Winter dormancy; BRE = Breeding and POST-B = Post-breeding. The body, testes and thyroid weights showed significant variation (p < 0.001) by ANOVA. Body = REC, WIN vs. QUI, BRE, POST-B; Testes = QUI vs. REC, WIN, BRE, POST-B; REC vs. BRE; WIN vs. REC, BRE; POST-B vs. REC, WIN, BRE; Thyroid = POST-B vs. QUI, REC, WIN, BRE; BRE vs. QUI, REC, WIN are statistically significant (P < 0.05). Values are mean ± S.E.
Fig. 3 — Thyroid follicular epithelial height (µm), thyroid colloid (mean areal fraction) and circulating thyroxine (ng/ml) concentration of male *S. heathi* during different reproductive stages. QUI = Quiescence; REC = Recrudescence; WIN = Winter dormancy; BRE = Breeding and POST-B = Post = breeding. The thyroid follicular epithelial height (p < 0.05), thyroid colloid (p < 0.01) and circulating thyroxine (p < 0.01) concentration showed significant variation by ANOVA. Thyroid follicular epithelial height = REC vs. QUI, WIN, BRE, POST-B; Thyroid colloid = POST-B vs. REC, WIN, BRE; Thyroxine = QUI vs. REC, WIN, BRE; POST-B vs. QUI, REC, WIN, BRE are statistically significant (P < 0.05) by Duncan’s. Values are mean ± S.E.
A positive relationship was observed between thyroid and testes from quiescence to winter dormancy, during this period changes in the body weight also showed a significant correlation with changes in the testicular weight and androstenedione synthesis. Whereas relationship between thyroid and testes was not positive during the phase from breeding to post-breeding. During this period the changes in the testicular activity was not correlated with the body weight. It is suggested that while comparing thyroid-gonadal relationship changes in the body weight (metabolic activity) and its inter-relationship with reproductive axis must be taken into consideration.

REFERENCES


SHI, Z. D. & BARRELL, G. K., 1992, Effects of thyroidec- 
tomy on seasonal patterns of live weight, testicular func-
tion, antler development and maiting in red deer stage. 
Springer Verlag, New York._

SINGH, K. & KRISHNA, A., 1995, Inhibitory effects of 
melatonin on testosterone but not on androstenedione 
production during winter in the vespertilionid bat, 

SINGH, K. & KRISHNA, A., 1996, Seasonal changes in cir-
culating serum concentration and _in vitro_ testicular se-
cretion of testosterone and androstenedione in the male 
vespertilionid bat, Scotophilus heathi. _J. Exp. Zool., 276_
: 43-52.

VELICKY, J. & TITLBACK, M., 1972, A study of the bat 
thyroid gland in winter and early spring. _Folia Morphol.,
20_: 406-415.

VELICKY, J. & TITLBACK, M., 1974, Electron microscopic 
observations in the thyroid gland of active bats. _Z. Mikrosk.

WEBSTER, J. R., MOENTER, S. M., WOODFILL, C. J. I. 
& KARSCH, F. J., 1991, Role of thyroid gland in seaso-
nal reproduction. II. Thyroxine allows a season specific 
suppression of gonadotropin secretion in sheep. _Endo-
crinology, 129_: 176-183.

WEIBEL, E. R., 1979, Stereological method, vol. 1. _Prac-
tical method for biological morphometry_, New York, Aca-
demic Press.

WEST, M. J., 1993, New stereological methods for count-

YOUNG, R. A., RAJATANAVIN, R., BRAVERMAN, L. E. 
& TENVANT, B. C., 1986, Seasonal changes in serum 
thyroid hormone binding proteins in the woodchuck 
(Marmota monax). _Endocrinology, 119_: 967-971.