



Study of seasonal histopathological changes in the Parathyroid gland of the smooth water snake, *Enhydris enhydris* (Schneider)

M.V. Kagwade

Department of Zoology, Elphinstone College, Mumbai 400032

Dr. Homi Bhabha State University

ABSTRACT

Enhydris enhydris is an inhabitant of India, Indonesia, Bangladesh, China and Malaysia found commonly in lakes, estuaries, marshes and wet fields. It is a viviparous snake that is an adaptation for aquatic habitat where parental care or protection of eggs is seldom possible. This non-poisonous snake is carnivorous, feeds on small fish and is thus an important component in the biodiversity of a lake occupying a higher trophic level.

Parathyroid gland is an important endocrine gland concerned with calcium metabolism. In a water snake like *E. enhydris* it is interesting to understand the histopathological behaviour of parathyroid which generally has a correlation with the environmental temperature, seasonal fluctuations of calcium in lake water and also breeding season.

Adult snakes were collected from the coast around Mumbai. They were maintained in tanks and acclimatised to laboratory conditions. Five males and five females were sacrificed under Nembutal anaesthesia on the same day and time every month throughout the year. The parathyroids were excised, fixed, processed in graded series of alcohol and paraffin embedding, sections cut at 6 μ and stained with hematoxylin-phloxin. Nuclear diameter of the cells was measured using an ocular micrometer.

The parathyroid glands show seasonal histological changes. The glands are more active in the months of November-December reflected by an increase in nuclear diameter of the cells. The nuclear diameter was lowest in the month of July and the gland also showed partial degeneration suggesting hypoactivity.

KEY WORDS

Parathyroid gland, *Enhydris enhydris*.

INTRODUCTION

The poikilotherms show marked seasonal changes in their habit and physiological activities. These are mainly related to thermoregulation, nutrition and reproduction. Those living in temperate climates hibernate during winter months while very hot dry seasons in the tropics are tided over by aestivation. The reptiles have a low metabolic rate and remain inactive for long periods except for feeding and breeding. The parathyroids of these animals show marked seasonal histological (Sidky,1965; Dubewar and Suryawanshi,1978a; Akbarsha,1983; Singh and Kar,1984; Swarup and Pandey, 1990b; Warbhuwan and Padgaonkar,1996a), histometric (Akbarsha,1985b) and ultrastructural (Isono et al., 1979) changes. Any changes in the histology of the gland should reflect on the levels of circulating calcium and inorganic phosphorus levels.

The parathyroid glands of many lizards show seasonal histological changes. Peters (1941) has reported changes in the parathyroid gland of *Lacerta viridis* during winter. In *Uromastix hardwickii* (Dubewar and Suryawanshi,1978a) the parathyroid gland is hyperactive during summer and shows degenerative changes during winter. An elevation in plasma calcium and inorganic phosphorus levels accompany this in summer and low levels in winter. Similar observations were also made in *Calotes versicolor* (Akbarsha, 1985b) and *Varanus flavescens* (Swarup and Pandey,1990b). In the snake, *Natrix piscator*, there is no significant seasonal variation in the physiological function of the gland (Singh and Kar, 1983b), while in another snake, *Eryx johnii*, the parathyroid gland is hyperactive in late summer and rainy season (Singh and Kar, 1983a). In *Acrochordus granulatus*, the parathyroid gland showed maximum activity in November and minimum in the month of July (Warbhuwan and Padgaonkar,1996a).

From the above, it is apparent that the parathyroid glands of different species respond differently to changes in season. Moreover, excepting in a few cases there are no reports of the correlation in the changes in the histological structure of the glands to the circulating levels of serum calcium and inorganic phosphorus. This study attempts to correlate the changes, if any, in the histology of the parathyroid glands with the serum levels of calcium and inorganic phosphorus in the snake, *Enhydryis enhydryis*.

MATERIALS AND METHODS

Adult snakes were collected from the nearby sea coast around Mumbai. The snakes were available between July and January, for seven months. Repeated attempts to procure the snakes between February and June were unsuccessful. The snakes after collection were kept in tanks containing seawater at room temperature (27-28° C) for 48 hours for acclimatization. Five male snakes and five female snakes were sacrificed under Nembutal anaesthesia (2.5mg/100g body weight) on the same day and time every month throughout the year. Blood samples from each snake were collected from the aortic arch, sera separated and analyzed for serum calcium and inorganic phosphorus according to methods described by Trinder (1960) and Gomorri (1942) respectively. The rostral parathyroids and the thymus glands were excised and fixed in Bouins fluid. After routine

processing in graded series of alcohol and paraffin embedding, sections were cut at $6\mu\text{m}$ and stained with hematoxylin-phloxin method. The nuclear diameter of the cells of the parathyroid were measured using an ocular micrometer. Each nucleus was measured along its long and short axis and its mean value was calculated. From each group male/female) 250 nuclei were randomly measured every month, i.e., 50 nuclei from each snake.

RESULTS

E. enhydris exhibits seasonal and sexual differences in the serum levels of calcium and inorganic phosphorus. The females have higher values of serum calcium annual mean ($14.94 \pm 1.71\text{mg}\%$) and inorganic phosphorus annual mean ($5.2 \pm 0.75\text{mg}\%$) as compared to those of males annual serum calcium mean ($12.40 \pm 0.63\text{mg}\%$) and inorganic phosphorus ($4.32 \pm 0.47 \text{mg}\%$). During the breeding season, from October to December, the females have significantly high values of serum calcium with the peak values in the month of December (Table 1). The minimum values are recorded in the month of July. As compared to females, the seasonal changes in the serum calcium and phosphorus levels are less significant (Table 1).

The nuclear diameter ranges between $2.90 \pm 0.17\mu\text{m}$ and $4.50 \pm 0.38\mu\text{m}$ in the male snakes and $2.25 \pm 0.25 \mu\text{m}$ in the female snakes, with an annual mean of $3.85 \pm 0.19 \mu\text{m}$ and $4.02 \pm 0.38 \mu\text{m}$ respectively. The nuclei are small in both sexes in the month of July. Thereafter, the size increases and becomes large in the month of December (Table 2). During this month, the levels of serum calcium and inorganic phosphorus in both female and male snakes also increases.

The parathyroid gland is composed of glandular parenchyma with a cord-like arrangement of cells separated by thin stroma of connective tissue (Fig1). These cells have inconspicuous cell boundaries with very little cytoplasm and oval or round nuclei.

The parathyroid gland of both sexes shows partial degenerative changes in the month of July (Figure 2 and Figure 3). The degenerative changes are conspicuous in the central part of the gland. The arrangement of the cells situated near the degenerating area is disturbed and they migrate towards the central space where they degenerate. The centre of the gland appears to be sparsely filled with a few cells in different stages of degeneration. The histology of the parathyroid during the other months of the year is normal.

DISCUSSION

In *E. enhydris*, significantly high serum calcium and inorganic phosphorus values were recorded in the month of December. The nuclear diameter of the parathyroid gland of both sexes also increased during this month, suggesting an increased activity of the gland. Similar observations were made in the snake *Acrochordus granulatus* (Warbhuwan and Padgaonkar,1996a). Further, the increase in serum calcium and inorganic phosphorus levels were more significant in females than males. Similar observations were made in *Uromastix*

hardwickii (Dubewar and Suryawanshi, 1978a), *Calotes versicolor* (Akbarsha,1985b) and *Varanus flavescens* (Swarup and Pandey, 1990b). During the breeding season the serum calcium and inorganic phosphorus levels in the females are higher as compared to males. The rise in the values in females during the months of November to December (breeding season) can be attributed to the endogenous secretion of estrogen. Other researchers have also drawn similar conclusions in other reptilian species (Dessauer et al., 1956; Dessauer and Fox, 1959; Clark, 1967b; Oguro and Uchiyama, 1972; Dubewar and Suryawanshi,1978a; Akbarsha,1983,1985; Swarup and Pandey,1990b). Similar observations were made in the snake *Acrochordus granulatus* (Warbhuwan and Padgaonkar,1996a).

The parathyroid glands in many reptiles showed hyperactivity during summer when the environmental temperature is high and hypoactivity in winter when the temperature is low (Peters,1941; Sidky, 1965; Dubewar and Suryawanshi, 1978a; Akbarsha,1985). In *E. enhydris*, higher activity of the parathyroid gland was observed during winter i.e., November through December which is also the breeding season. Further in July, during monsoon when the temperature is moderately high, the parathyroid gland showed signs of hypoactivity and low levels of serum calcium and inorganic phosphorus. It can thus be concluded that the activity of parathyroid gland in *E. enhydris* does not depend on environmental temperature.

Partial degeneration of the parathyroid gland in *E. enhydris* is similar to that observed in the lizard, *Uromastix hardwickii* (Dubewar and Suryawanshi,1978a). Parts of the gland appeared to be normal, suggesting that the gland is not completely degenerated. The nuclear diameter of the cells of the parathyroid during this month, i.e. July, was at the minimum measuring $2.25\pm 0.25\mu\text{m}$ in females and $2.90\pm 0.17\mu\text{m}$ in males. The serum calcium and inorganic phosphorus levels was at the minimum suggesting that the parathyroids are hypoactive.

Thus, it can be concluded that in the snake *E. enhydris* the levels of serum calcium and inorganic phosphorus are related to the activity of the parathyroid gland as well as the reproductive cycle.

REFERENCES

- Akbarsha, M.A. 1983. Seasonal and sexual differences in the effects of parathyroidectomy in the Indian garden lizard, *Calotes versicolor* with a note on follicle formation in reptilian parathyroids. *Amphibia-Reptilia* 4:185-194.
- Akbarsha, M.A.1985b. Seasonal-morpho and histometric changes in the parathyroids of the lizard, *Calotes versicolor* (Daudin). *Z. mikrosk-anat. Forsch* 99 6: 929-936.
- Clark, N.B.1967b. Influence of estrogens upon serum calcium, phosphate and protein concentrations of fresh water turtles. *Comp. Biochem. Physiol.* 20: 823-834.

Dessauer, H.C., Fox, W. and Gilbert, N.L. 1956. Plasma calcium, magnesium and protein of viviparous colubrid snakes during estrous cycles. Proc. Exp. Biol. Med. 92:299-301.

Dessauer, H.C and Fox, W.1959. Changes in ovarian follicle composition with plasma levels of snakes during estrus. Am.J.Physiol. B197(2): 360-366.

Dubewar, D.M., Suryawanshi, S.A. 1978a. Seasonal variations in the parathyroid gland of the lizard *Uromastix hardwickii* (Gray). Z. mikrosk-anat. Forsch (Leipzig). 92(2): 298-304.

Isono, H., Shoumura, S., Ishizaka, K. Hayashi, K. and Yamahira T. 1979. Ultrastructure of the parathyroid gland of the Japanese lizard (*Takydromis tachydromoides*) in the spring and summer season. J. Morphol. 161(2): 145-148.

Oguro, C. and Uchiyama, M. 1972. Effect of exogenous estrogen on serum calcium concentration in the snake, *Rhabdophis tigrinus tigrinus*. Annot.Zool. Jpn. 45: 214-217.

Sidky, Y.A.1965. Histological studies on the parathyroid gland of lizards. Z. Zellforsch. Mikrosk. Anat. 65:760-769.

Singh, R. and Kar, I. 1983a. Parathyroid and ultimobranchial glands of the sand boa *Eryx johnii*. Gen. Comp. Endocrinol. 51(1): 66-70.

Singh, R. and Kar, I. 1983b. Parathyroid gland of freshwater snake *Natrix piscator*. Gen. Comp. Endocrinol. 51(1): 71-76.

Swarup, K. and Pandey, A.K.1990b. Seasonal changes in the parathyroid gland of the yellow monitor, *Varanus flavescens* Gray, in relation to the serum calcium and inorganic phosphorus levels. Proc. Nat. Acad. Sci., India. 60B: 229-236.

Warbhuwan, A.P. and Padgaonkar, A.S. 1996a. Seasonal changes in the parathyroid gland of the estuarine snake, *Acrochordus granulatus* (Schneider) in relation to the serum calcium and inorganic phosphorus levels. Trends in Life Sciences (India). 11(1):7-13.

TABLE 1: Seasonal changes in the serum calcium and inorganic phosphorus levels in the female and male snake, *Enhydris enhydris*

MONTH	SERUM CALCIUM mg%		SERUM INORGANIC PHOSPHORUS mg%	
	Female	Male	Female	Male
JULY	10.98 ± 0.25	10.48 ± 0.25	3.66 ± 0.17	3.01 ± 0.16
AUGUST	11.27 ± 0.22	10.96 ± 0.63	3.70 ± 0.28	3.51 ± 0.12
SEPTEMBER	12.66 ± 0.27	11.23 ± 0.22	4.03 ± 0.20	3.53 ± 0.12
OCTOBER	12.30 ± 0.80	11.38 ± 0.36	4.48 ± 0.19	4.22 ± 0.31
NOVEMBER	17.45 ± 0.39	13.39 ± 0.25	7.94 ± 0.50	6.25 ± 0.11
DECEMBER	24.74 ± 1.13	14.86 ± 0.53	8.71 ± 0.77	6.34 ± 0.28
JANUARY	15.18 ± 0.41	14.55 ± 0.36	3.91 ± 0.17	3.78 ± 0.22
ANNUAL MEAN	14.94 ± 1.71	12.40 ± 0.63	5.20 ± 0.75	4.32 ± 0.47

TABLE 2: Seasonal changes in the nuclear diameter of the parathyroid cells in the male and female snake, *Enhydris enhydris*.

MONTH	NUCLEAR DIAMETER	NUCLEAR DIAMETER
	μm Male	μm Female
JULY	2.90 ± 0.17	2.25 ± 0.25
AUGUST	3.50 ± 0.38	2.90 ± 0.46
SEPTEMBER	4.17 ± 0.15	3.80 ± 0.25
OCTOBER	4.22 ± 0.20	4.50 ± 0.49
NOVEMBER	3.50 ± 0.53	4.90 ± 0.12
DECEMBER	4.17 ± 0.44	5.30 ± 0.35
JANUARY	4.50 ± 0.38	4.50 ± 0.25
ANNUAL MEAN	3.85 ± 0.19	4.02 ± 0.38

FIGURE 1: Photomicrograph of the parathyroid gland showing cord like arrangement of cells. 250X

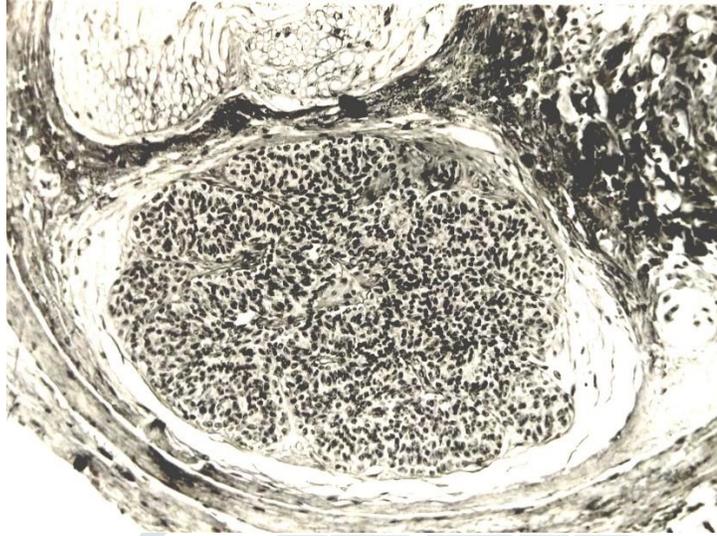


FIGURE 2: Photomicrograph of the parathyroid gland of the male snake showing partial degeneration in the month of July. 250X.

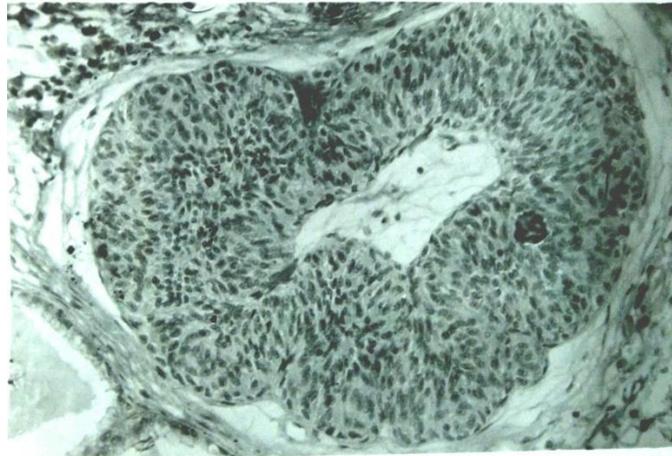


FIGURE 3: Photomicrograph of the parathyroid gland of the female snake showing partial degeneration in the month of July. 250X.

