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Parathyroid and Ultimobranchial Glands of the Fresh Water Snake, *Amphiesma stolata* (Linn.)

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Anita P. Warbhuwan and Arun S. Padgaonkar (1993) Parathyroid and ultimobranchial glands of the fresh water snake, *Amphiesma stolata* (Linn.). *Bull. Inst. Zool., Academia Sinica* **32**(3): 157-161. The fresh water snake *Amphiesma stolata* possesses two pairs of parathyroid glands (PTGs), one pair lying on each side of the head. The caudal PTG pair is situated on the inner margins of the lower thymus lobes, while the rostral pair is situated near the hinges of the jaws. The parathyroid glands are surrounded by connective tissue capsules, and are composed of cell cords of a single cell type. The glandular parenchyma is well-vascularized.

In addition, *A. stolata* has a single pair of ultimobranchial glands (UBGs). The right UBG is situated between the upper and lower thymus lobes, while the left UBG lies at the inner margin of the upper thymus lobe. Each gland is covered with a thick connective tissue capsule and consists of compact cell clumps and cords which are well-vascularized.

Key words: Parathyroid and Ultimobranchial glands, Snake, Buff-striped keelback.

There have been very few histomorphological studies made on the parathyroid glands (PTGs) of reptiles, especially snakes. Reptiles possess one or two pairs of parathyroid glands, depending upon the group or species. The presence of additional smaller parathyroid glands has occasionally been reported; previously, these have been regarded as accessory PTGs (Adams 1939, Peters 1941, Oguro and Sasayama 1976, Singh and Kar 1983a 1983b). Confusion still exists regarding the number of parathyroids in some reptilian species, as various epithelial glands described by earlier authors were sometimes questionably interpreted as being parathyroid tissue (Clark 1970). Similarly, very few studies have been made on the ultimobranchial glands (UBGs) of reptiles. A survey of the literature reveals many discrepancies regarding the UBGs of reptiles, especially snakes. These findings encouraged us to undertake an histomorphological study of the parathyroid and ultimobranchial glands of the Buff-striped keelback snake, *Amphiesma stolata*.

MATERIALS AND METHODS

Male and female specimens of *A. stolata* were captured in Bombay suburbs, then taken to the lab and dissected under Nembutal anaesthesia (2.5 mg/100 gm body wt.). PTGs and UBGs were located with the help of a dissecting microscope, excised, and fixed in Bouin's fluid. After paraffin embedding, 5 sections were cut and stained

with haematoxylin and phloxin.

RESULTS

Parathyroid glands

A. stolata has two pairs (rostral and caudal) of parathyroid glands (PTGs), one pair lying on each side of the head. The caudal PTGs are situated on the inner margins of the lower thymus lobes (Figs. 1 and 2). Rarely, they are pushed back into the thymus tissue (Figs. 2 and 3). The right rostral pair is situated at the bifurcation of the carotid artery, while the left pair is situated at the bifurcation of the jugular vein (Fig. 1).

The sizes of both pairs of glands (RPTGs and CPTGs) are almost the same. In their natural condition, the glands are whitish in color and bead-like in appearance.

Each parathyroid gland is surrounded by a connective tissue capsule (Fig. 3). The glands are composed of cell cords of a single type of cell, with connective tissue and capillaries lying between the cords. Cell boundaries are generally inconspicuous. The nuclei are round or oval in shape with conspicuous nucleoli and chromatin granules. The cells contain very little phloxinophilic cytoplasm.

Ultimobranchial glands

A. stolata have one pair of ultimobranchial glands (UBGs). The right UBG is situated in between the upper and lower thymus lobes (Fig. 4), while the left UBG lies on the inner margin of the upper thymus lobe (Fig. 1). Both glands are equal in size; in their natural condition they are translucent.

Each ultimobranchial gland is covered with a thick connective tissue capsule (Fig. 5). The glands are vascularized and made up of cell cords and clumps (Fig. 5).

DISCUSSION

The presence of two pairs of PTGs in *A. stolata* coincides with observations made in other ophidian species (Saint Remy and

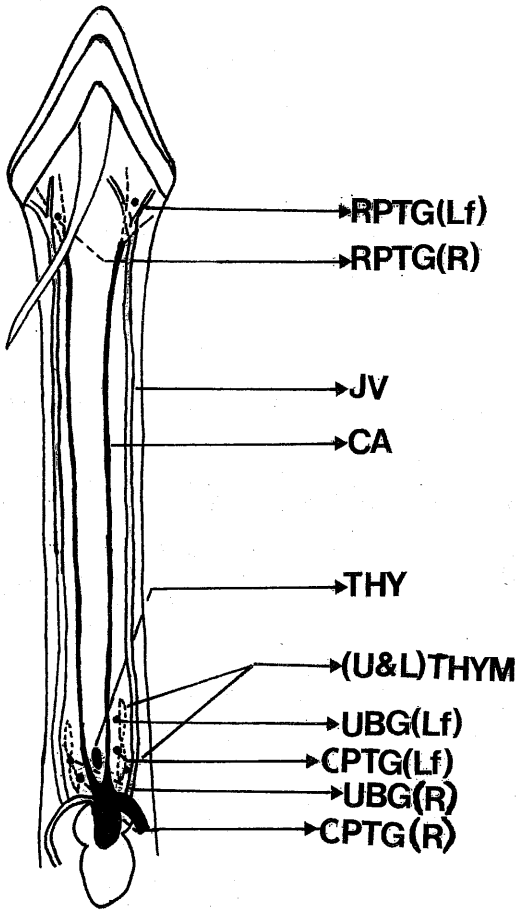

Fig. 1. Anatomical locations of the pharyngeal endocrine glands of the snake, *Amphiesma stolata*.
CPTG – Caudal parathyroid glands
RPTG – Rostral parathyroid glands
UBG – Ultimobranchial glands
U – Upper
L – Lower
THYM – Thymus
JV – Jugular vein
CA – Carotid artery
R – Right
Lf – Left
Thy – Thyroid

(Magnification two times larger than *in vivo*)

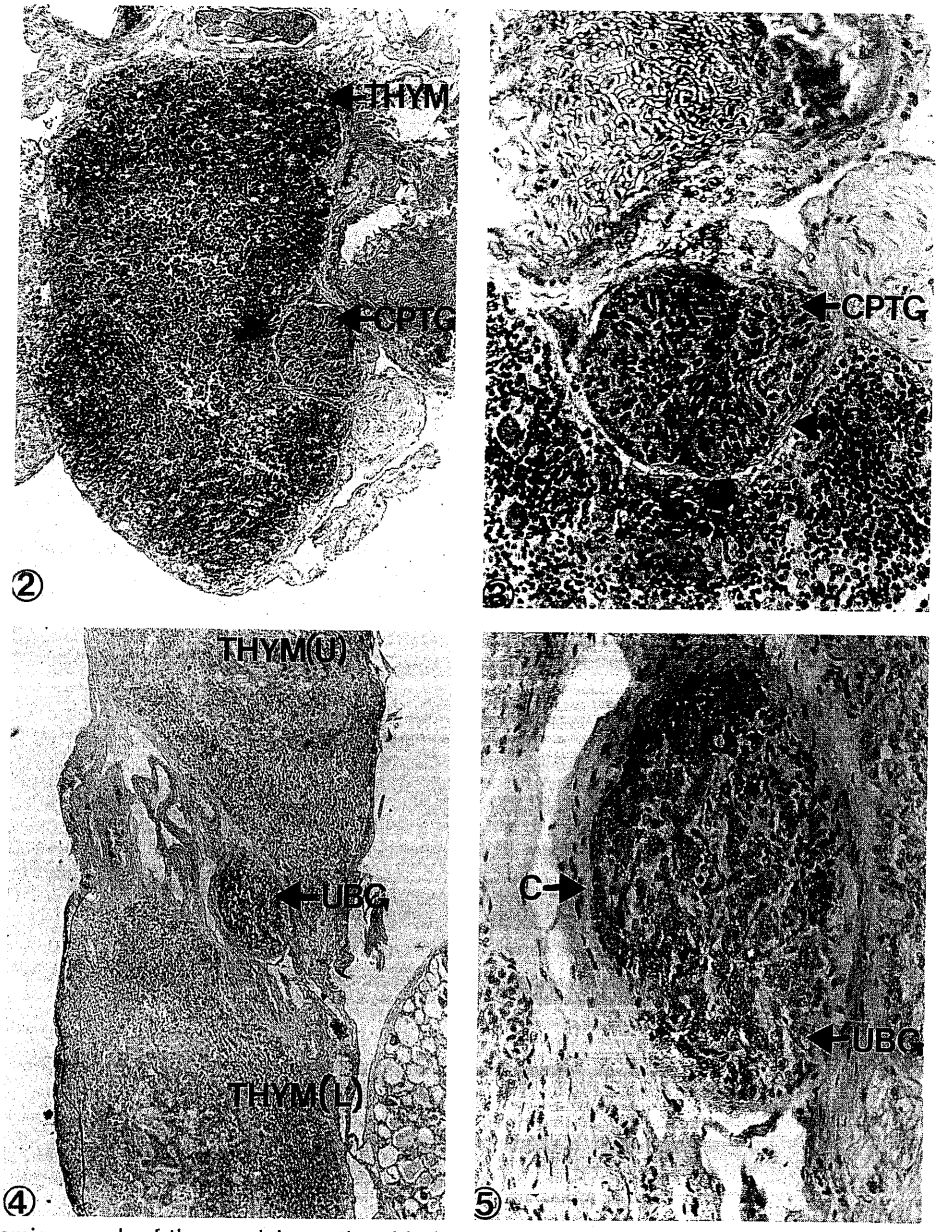

Fig. 2. Photomicrograph of the caudal parathyroid gland of the snake, *A. stolata*, showing its disposition. x100
THYM – Thymus
CPTG – Caudal parathyroid gland

Fig. 3. Photomicrograph of the caudal parathyroid gland of the snake, *A. stolata.* x250
C – Capsule
CPTG – Caudal parathyroid gland

Fig. 4. Photomicrograph of the ultimobranchial gland of the snake, *A. stolata.* x100
THYM – Thymus
UBG – Ultimobranchial gland
U – Upper
L – Lower

Fig. 5. Photomicrograph of the ultimobranchial gland of the snake, *A. stolata* under high magnification. x400
C – Capsule
UBG – Ultimobranchial gland

Prenant 1904, Harrison and Denning 1929, Neudeck 1969, Clark 1970, Oguro 1970, Singh and Kar 1983a 1983b 1984, Alcobendas 1989, Padgonkar and Warbhuwan 1991). The locations of the *A. stolata* PTGs agree with those reported by Herdson (1956).

The accessory parathyroid tissue, which has been reported as being present in some ophidian species (Clark 1979, Singh and Kar 1983a 1983b), is absent in *A. stolata*.

The histological features of the parathyroid glands of *A. stolata* are: connective tissue capsule, blood capillaries, and a glandular parenchyma which is made up of cell cords of a single type of cell. These histological features compare favorably with those of other ophidian species (Clark 1971, Singh and Kar 1983a 1983b 1984, Padgaonkar and Warbhuwan 1991). The parathyroid gland of some snakes have been shown to contain follicles (Clark 1971, Oguro 1970 1972, Singh and Kar 1983a), while those observed in other species contain large vesicles (Singh and Kar 1983a 1984). Follicles are absent from *A. stolata.* Similar observations have also been made for another fresh water snake, *Natrix piscator* (Singh and Kar 1983b).

The occurrence of a single pair of ultimobranchial glands in *A. stolata* is in agreement with observations made in other ophidian species (Watzka 1933, Sehe 1965, Yoshihara *et al.* 1979, Singh and Kar 1982 1983a 1983b 1985, Alcobendas 1989, Padgaonkar and Warbhuwan 1991). In contrast, only the left UBG has been reported as being present in some ophidians (Francescon 1929). The bilateral location of same-sized ultimobranchial glands in the thyro-thymic region of *A. stolata* generally agrees with the findings of Yoshihara *et al.* (1979), Singh and Kar (1982 1983a 1983b 1985), and Padgaonkar and Warbhuwan (1991). On the other hand, in the snake *Eryx johni* these glands are located midway between the rostral and caudal pairs of parathyroid glands (Singh and Kar 1983a). Some researchers (Francescon 1929, Watzka 1983, Sehe 1965) previously reported that the left UBG is larger than the right.

The histological features of the UBG of *A. stolata* are similar to those reported in other ophidian species (Yoshihara *et al.* 1979, Singh and Kar 1982). Follicles were not observed. The ciliated and goblet cells in the follicular epithelium, as reported in some lizards and terrestrial snakes (Das and Swarup 1975, Anderson and Capen 1976, Yoshihara *et al.* 1979, Singh and Kar 1983a 1985, Padgaonkar and Warbhuwan 1991) are absent in *A. stolata.* Singh and Kar (1982) made similar observations in another fresh water snake, *Natrix piscator.*

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印度水蛇(*Amphiesma stolata*)副甲狀腺與後鰓腺之形態研究

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印度淡黃條紋平背水蛇（*Amphiesma stolata*）具有二對副甲狀腺，分列於身體的兩側。靠近尾端的一對副甲狀腺位於下葉胸腺的內緣，而靠近頭部的這對腺體則位於頸靜脈的分叉處。副甲狀腺是由單一種細胞構成，腺體外有一層結締組織包覆，其軟體細胞組織內則廣布了毛細管（動脈毛細管、靜脈毛細管及淋巴毛細管）。

此種水蛇另外具有一對後鰓腺，右側的後鰓腺是位於上下葉胸腺之間，而左側之腺體則位於上葉胸腺的內緣。此腺體也被一層厚厚的結締組織包被，它是由細胞塊及排列成索狀之細胞構成，腺體上也廣布了毛細管。

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Expression of Rainbow Trout Growth Hormone cDNA in Yeast

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Huai-Jen Tsai, Chun-Feng Tseng and Tsong-Teh Kuo (1993) Expression of rainbow trout growth hormone cDNA in yeast. *Bull. Inst. Zool., Academia Sinica* **32**(3): 162-170. An episomal expression plasmid with an insert of rainbow trout growth hormone (rtGH) cDNA under the control of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter was constructed. The resultant plasmid was harbored by *Saccharomyces cerevisiae* 20B12 (designated as strain Y-105) and maintained in amounts of around 20-25 copies per cell. This yeast-produced recombinant GH is a fusion protein in which the mature rtGH (188 amino acids) was preceded by six deduced amino acids: Met-Gly-Gln-Gly-Ala-Ala. Northern blotting showed that the rtGH cDNA was transcribed into mRNA in yeast cells. A 22 kilo-daltons (kDa) protein band was detected in the soluble fraction of cellular proteins extracted from the Y-105 strain. This band was found to be immunoreactive to an antiserum raised against natural chum salmon growth hormone.

Key words: Glyceraldehyde-3-phosphate dehydrogenase promoter, Expression plasmid, Immunoreaction, Recombinant growth hormone.

Growth hormone (GH) is a polypeptide hormone produced by proximal pars distalis cells of the anterior pituitary gland which regulates growth and metabolism in vertebrates (Ganong 1983). GH enhances appetite, feed efficiency, and growth rate in fish (Donaldson 1979); thus, GH may be the most promising agent for growth promotion in aquaculture (Zohar 1989). However, the availability of natural GH is extremely limited, since its preparation from fish pituitary glands is cost-prohibitive.

Most fish GH cDNA have been expressed in *Escherichia coli* (Sekine *et al.* 1985, Agellon and Chen 1986, Saito *et al.* 1988, Rentier-Delrue *et al.* 1989, Sato *et al.* 1989, Tsai *et al.* 1993). However, the greatest disadvantage of this expression system is that the biosynthesized GH accumulates within the inclusion body in a denatured and unmodified form. As a result, this recombinant GH (rGH) has to be processed in a complicated and expensive way in order to renature it to its native form before it can be used.

Saccharomyces cerevisiae is considered a better host cell for expressing GH cDNA because: (a) it is capable of processing post-translational modification; (b) the gene product exists in a natural form; (c) it costs less for culturing than tissue culture; and, (d) it