

## Modulations of thyroid activity following reproductive hormonal manipulations in soft-shelled turtles, *Lissemys punctata punctata*

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Prolactin and sex hormones are known to modulate thyroid activity in vertebrate animals, but their findings are different in different vertebrates. Prolactin inhibits thyroid activity in higher dose by decreasing <sup>131</sup>I uptake, serum PBI and T<sub>4</sub> levels, follicular size and epithelial height in mammals, birds, red efts *Triturus viridiscens*, prometamorphic tadpoles of *Rana pipiens* and *Rana catesbeiana*, but stimulates in low dose by increasing values of these parameters in mammals, birds, crested newts and eels (Decuyper and Kühn, 1985). Estradiol, estriol, estrone and progesterone also inhibit thyroid activity in intact/gonadectomized/hypophysectomized rats, rabbits and rhesus monkeys (Boado et al., 1983), intact juvenile ducks or ovariectomized Japanese quail (Maiti and Sahu, 1982; Pethes and Peczely, 1983) and in rainbow trout *Salmo gairdneri*, *Anabus testudineus* and *Chana punctatus* (Leatherland, 1985; Cyr et al., 1988), but stimulate in intact/gonadectomized/hypophysectomized rats and other mammals (Bisaria and Kapoor, 1976). Thyroid activity is not altered after progesterone treatment in rabbit, or estradiol or TSH treatment in adult male rats (Kannan et al., 1980). Whereas combined treatments of progesterone and estradiol, or progesterone, estradiol and testosterone inhibit thyroid activity in adult male rats, female ducklings and ovariectomized Japanese quail, but stimulates in adult male and female rats (Maiti and Sahu, 1982; Pethes and Peczely, 1983).

Contrary to the largely inhibitory action of prolactin and sex hormones on thyroid activity in most of the vertebrates studied, these hormones have also been reported to stimulate thyroid activity in a species of turtle (*Clemmys caspica leprosa* and other reptiles *Anolis carolinensis*) (Combescot, 1956; Licht and Jones, 1967). Role of sex hormones on thyroid activity is extremely negligible in reptiles especially in turtles. In the current article, this problem was resolved in turtles.

Juvenile female soft-shelled turtles, *Lissemys punctata*, collected from natural populations near Calcutta, were selected for the present experiments, since their endogenous milieu of reproductive hormones was anticipated to be lower than that of adults (Sen and Maiti, 1988). Consequently changes in thyroidal activity after hormonal treatments could be largely due to exogenous hormones. Animals were kept in the aquaria (150 cm × 90 cm × 60 cm) in small groups (4 each) in order to avoid effects due to crowding stress. Turtles were maintained in controlled laboratory conditions (temperature, 25°C and photoperiod, 12L: 12D). Food (shrimps and earthworms) was easily accessible *ad libitum* to the turtles throughout the experiments. Turtles were acclimatized to the laboratory conditions for 5 days prior to experiments. Forty specimens were divided into 10 groups of 4 each. Group I served as control for all the experiments. Out of 9 groups, 3 groups each were treated with hormones: (groups II, III & IV for prolactin; V, VI & VII for estrogen and VIII, IX & X for progesterone). Each hormone was injected intramuscularly (in the hind legs of turtles) in three different doses (25 µg, 50 µg or 100 µg per 100 gm body wt once daily for 10 days) to 3 different groups of turtles. Prolactin (LTH No. L-7009, Sigma, USA) and estrogen (17β-estradiol Batch no. SO-6429) and progesterone (Pregnenolone Batch no. 3-Sp-813), both from Scherring Corporation, USA were used. Turtles were anaesthetized to death with chloroform at a particular time of the day (10 a.m.) in order to avoid effects due to circadian rhythm of thyroid activity (Choudhury et al., 1982). Body weights of turtles were recorded prior to autopsy. Thyroid glands were quickly dissected out, weighed on a torsion balance (ROLLER-SMITH, USA) and immersed in Bouin's fixative for histological study. Following routine microtomy, 5 µm thick paraffin sections, cut by

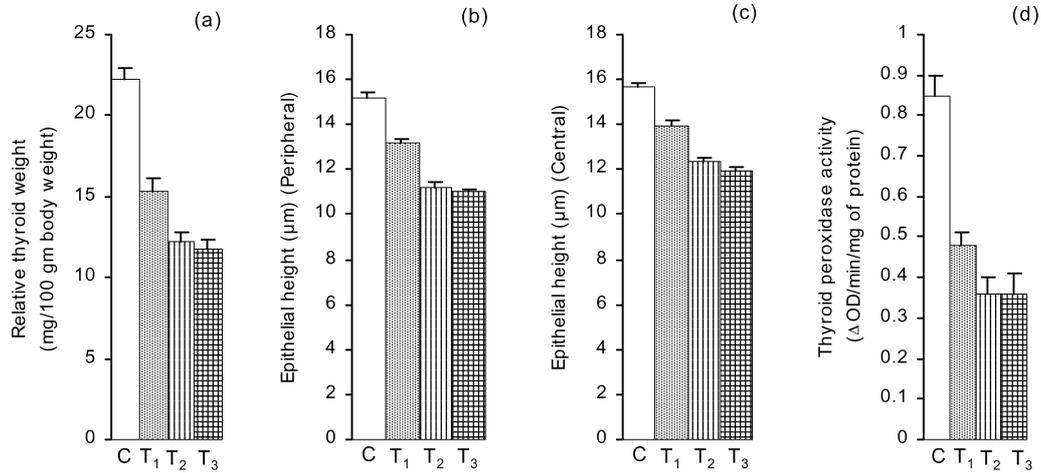
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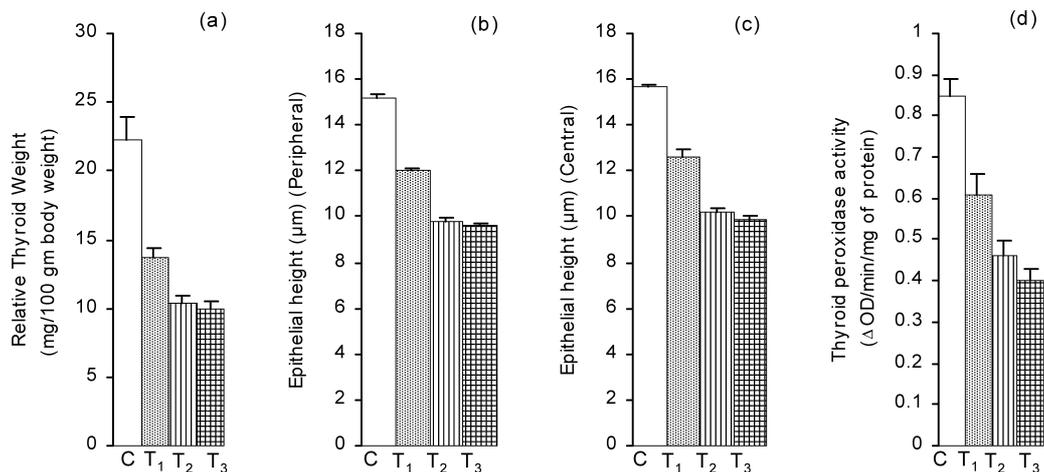
microtome (BIOCUT, Cambridge), were stained with Masson's trichrome technique. Thyro-follicular epithelial height was measured ( $\mu\text{m}$ ) by ocular micrometer from peripheral and central regions of the gland. At least 20 follicles each for the peripheral and central regions of the gland per section were studied from 10 widely separated random sections of the gland of each specimen. Thyroid peroxidase activity was measured (Bhattacharya and Datta, 1971) using O-dianisidine as hydrogen donor by a spectrophotometer (BECKMAN) at 460 nm wavelength with 1 cm path of light. Protein content was measured by the method of Lowry et al. (1951) using bovine albumin as standard. All the data were analysed statistically by ANOVA (Snedecor and Cochran, 1971).

### Control

The relative weight of the thyroid gland of control animals is presented in figs 1a, 2a, 3a. The thyroid gland consists of numerous follicles, each lined by a single layer of short columnar cells with partially heterogeneous colloid materials stored in both the peripheral (fig. 4) and central follicular lumens. There is no significant difference in the cell size or epithelial height between the peripheral and central follicles (figs 1b & c, 2b & c, 3b & c). Thyroid peroxidase



**Figure 1.** Histograms showing decreased relative thyroid weight (a), follicular epithelial height (peripheral: b, central: c) and peroxidase activity (d) after prolactin treatments in different doses in turtles. (C: Control; T<sub>1</sub>: 25  $\mu\text{g}$ , T<sub>2</sub>: 50  $\mu\text{g}$ , T<sub>3</sub>: 100  $\mu\text{g}$  each per 100 gm body wt to 3 different groups of turtles daily for 10 days) (ANOVA:  $P < 0.01$ ).



**Figure 2.** Histograms showing decreased relative thyroid weight (a), follicular epithelial height (peripheral: b, central: c) and peroxidase activity (d) after estrogen treatment in different doses in turtles. (For other legends see fig. 1) (ANOVA  $P < 0.01$ ).