

Roles of Thyroid, Adrenal and Pancreatic Hormones on Thyroid Activity of the Soft-shelled Turtles *Lissemys punctata punctata* Bonnoterre

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The effects of some exogenous peripheral hormones (thyroxine, corticosterone, epinephrine, norepinephrine and insulin) on thyroid activity were investigated in juvenile female soft-shelled turtles, *Lissemys punctata punctata*. Each hormone was injected in three different doses (25 µg, 50 µg or 100 µg each per 100 g body weight, once daily at 9 AM) for 10 consecutive days. Thyroid activity was evaluated by gravimetry, histology (epithelial height) and thyroperoxidase assay. The findings revealed that thyroxine in low dose (25 µg) stimulated thyroid activity by increasing the relative thyroid weight, epithelial height and thyroperoxidase activity, but inhibited gland activity at a high dose (100 µg) by decreasing the values of all these parameters. The medium dose (50 µg) had no significant effect. All other hormones, in all doses, significantly decreased thyroid activity by decreasing the values of all the parameters. Thyroid responses to exogenous hormones are generally dose-dependent in turtles. The mechanisms of actions of the hormones administered are suggested.

Key words: Thyroxine, corticosterone, epinephrine, norepinephrine, insulin, thyroid, turtles.

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Information concerning the role of thyroxine on thyroid activity is rather negligible. Exogenous T₃ or T₄ inhibits thyroid activity in adult and neonatal rats, and common voles (DOBROWOLSKA *et al.* 1976; CSABA & NAGY 1986), and suppressed ¹³¹I uptake in white Leghorn cockerels or thyroid activity in *Lacerta* and *Gekko* (CHIU *et al.* 1967).

AAKERMAN and others (1961) have reported that cortisone, deoxycorticosterone or hydrocortisone significantly suppress iodine collection, iodine trapping by the thyroid, the capacity of the thyroid to concentrate radioiodine, thyroid epithelial percentage and the rate of ¹³¹I release in rats and rabbits. Hypothyroidism occurs following cortisone treatment in massive doses in rats. TSH infusion for 3 hours rapidly accelerates thyroid hormone release but simultaneous administration of cortisol and TSH inhibits the accelerated release of thyroid hormones in rats. Adrenalectomy increases thyroid weight, epithelial height, PBI level and ¹³¹I uptake in rats (GRUNT & CUNNINGHAM 1965). ACTH, deoxycorticosterone or hydrocort-

sone reduce ¹³¹I uptake with hypoactivity of the thyroid gland in rabbits (MIKHAILOV 1966). ACTH decreases T₃ and T₄ levels in piglets (DRO-RAK & NEUMARNOVA 1987). In contrast, hydrocortisone can stimulate thyroid gland activity by increasing epithelial height or secretory activity in the adrenalectomized male rats (MALENDOWICZ *et al.* 1974; MALENDOWICZ & FILIPAC 1975). ACTH and dexamethasone reduce plasma T₃ and T₄ levels in domestic fowl (MITCHELL *et al.* 1986), T₃ and rT₃ levels in *Gallus domesticus*, or T₃ level in broiler fowl (BUYSE *et al.* 1986). In fish (female European eels, *Anguilla anguilla*) cortisol injection decreases plasma T₄ and T₃ levels (REDDING *et al.* 1986), but cortisol or dexamethasone has no effect on plasma total thyroid hormone levels or plasma T₃/T₄ ratio in the rainbow trout, *Salmo gairdneri* (LEATHERLAND 1987).

JOASOO and MURRAY (1974a,b) have reported that epinephrine injection decreases ¹³¹I uptake and serum PBI level in intact or adrenalectomized rats or decreases the incorporation of ¹³¹I into iodo-

thyronine and ^{131}I uptake (over 24 hrs) by the thyroid gland in a dose related manner in rats. Epinephrine and norepinephrine significantly inhibit the TSH stimulated T_4 level in the thyroid gland of mouse *in vitro* (MAAYAN *et al.* 1981; TAKAMURA *et al.* 1982). But in dogs, intravenous injection of epinephrine increases the PBI^{131} level, attributable to the elevations of both T_3 and T_4 levels. In both juvenile and adult pigeons epinephrine and norepinephrine in lower dosage have no effect on the thyroid, but in higher dosage stimulate the thyroid gland histologically (BHATTACHARYA 1971). In contrast to the latter report, in Arctic Charr, *Salvelinus alpinus*, acute depression of plasma T_4 level occurs following higher doses of epinephrine and norepinephrine treatments (EALES *et al.* 1986).

KUMARESAN and TURNER (1966) have shown that insulin injection (0.99 μg to 1.22 $\mu\text{g}/100$ g body weight daily) increases the thyroid hormone secretion rate for shorter duration or even 8-14 days after withdrawal of the hormone treatment in rats. Long-term insulin treatment with successive increase of dose also increases ^{131}I uptake in rats, but not in rabbits (CHRISTEL *et al.* 1970). In fetal rat thyroid culture, insulin enhances ^{125}I incorporation into bound iodothyronines and tyrosines (NATAF *et al.* 1972). Insulin administration in rats, kept on a low iodine diet and KClO_4 , or on KClO_4 and 6-propyl-2-thiouracil, increases thyroid weight compared to the rats kept on goitrogens alone (JOLIN *et al.* 1974). Insulin increases serum TSH and T_3 levels in female rats (LEUNG *et al.* 1975) or thyroid hormone level in dogs (BLUM *et al.* 1977). Contrasting results are available in birds. Insulin injection decreases plasma T_3 and T_4 levels concomitant with a profound hypoglycemia in fed and fasted birds, and the effect is more pronounced in fasted than fed birds (MITCHELL & RAJA 1986).

The literature available indicates that thyroid, adrenocortical and adrenomedullary hormones have generally inhibitory actions on thyroid activity, but pancreatic insulin has a stimulating effect on thyroid gland in most of the homeothermic vertebrates studied. Information on the hormonal influence of thyroid activity in poikilotherms is scarce, especially in turtles. In the current article this problem was resolved in the soft-shelled turtle.

Material and Methods

Juvenile female turtles, *Lissemys punctata punctata*, Bonnoterre, were collected in March from wild populations near Calcutta. Female specimens were selected for the present investigation as they were abundantly available in March. Juvenile soft-shelled turtles (body weight ranging between

300-350 g) were used since their endogenous hormonal milieu of the ovary (evaluated from bioassay of its targets) (SEN & MAITI 1988), adrenal cortex and adrenal medulla (RAY & MAITI 1987) was lower than in adults. Sixty four specimens were divided in 16 groups of 4 each. They were kept in small groups to avoid effects due to population stress (DE *et al.* 1974). Animals were acclimatized to the controlled laboratory conditions (temperature, 25°C and light, 12L : 12D) in aquaria (150 cm x 90 cm x 60 cm) for 5 days prior to experiments. Hormones were injected intramuscularly into the hind legs of turtles on alternate days for 10 days (Table 1). All the experiments were terminated on day 11 and thyroid glands were dissected from the anaesthetized specimens by sodium barbital injection at a particular time of the day to avoid effect due to diurnal rhythm (CHOWDHURY *et al.* 1982).

Body weight was recorded prior to autopsy. Thyroid glands were weighed on a torsion balance (Roller-Smith, U.S.A.), immersed in Bouins fluid and processed for histological study. Five μm thick paraffin sections were prepared using a BIOCUT microtome (Cambridge Instruments, England) and stained by the Masson's trichrome technique. The epithelial height of the peripheral and central follicles of the thyroid gland was measured by binocular in μm . At least twenty follicles each for the peripheral and central regions of each section were considered. Ten widely separated sections were studied for each specimen. Thyroperoxidase activity was assayed following the method of BHATTACHARYA & DATTA (1971). Thyroid glands were homogenized in 0.05M phosphate buffer (pH 7.0); the homogenates were subjected to differential grades of centrifugation (Sorvall OTD-50, USA) at 105,000 g and the soluble supernatant fractions were assayed for peroxidase activity using 350 μmol of hydrogen peroxide as substrate, 0.1M phosphate buffer (pH 7.0) and 1% *O*-dianisidine in methanol as the hydrogen donor. 0.1 ml of Orthodiansidine dye was added to 6 ml of substrate-phosphate buffer. Subsequently, 2.9 ml of the latter solution was transferred to a test cuvette and the remainder was poured into a control cuvette. At zero time 0.1 ml of enzyme sample was added into the cuvette and mixed. OD was recorded at 420 nm in a Perkin-Elmer spectrophotometer using a 1 cm light path. OD was recorded at 15 s intervals for 2 min and the rate of change of enzyme activity per minute per mg of protein was calculated. The protein was estimated by the method of LOWRY *et al.* (1951).

All the data were analysed by a one-way analysis of variance (ANOVA) followed by Students' *t*-test (SNEDECOR & COCHRAN 1971).

Table 1

Experimental Protocol

Groups	Hormones	Dose and duration of treatment for all the hormones ($\mu\text{g}/100\text{ g}$ body weight daily for 10 days)
I : Control	Vehicle	— — —
Treated (II, III & IV)	Thyroxine (L-thyroxine, 3,3',5,5' tetraiodo-L-thyroxine (Sigma, USA, LOT No. T-2376)	T ₁ : 25 μg T ₂ : 50 μg T ₃ : 100 μg
Treated (V, VI & VII)	Corticosterone (Δ^4 -pregnene-11 β -21-diol-3,20-dione, Sigma, USA)	Same
Treated (VIII, IX & X)	Epinephrine (Adrenaline bitartrate, Sigma USA, LOT No. E-4375)	Same
Treated (XI, XII & XIII)	Norepinephrine (Arterenol bitartrate crystalline, (Sigma, USA, (of No. A-9512)	Same
Treated (XIV, XV & XVI)	Insulin (Bovine pancreas crystalline, Sigma, USA, (No. I-5500)	Same

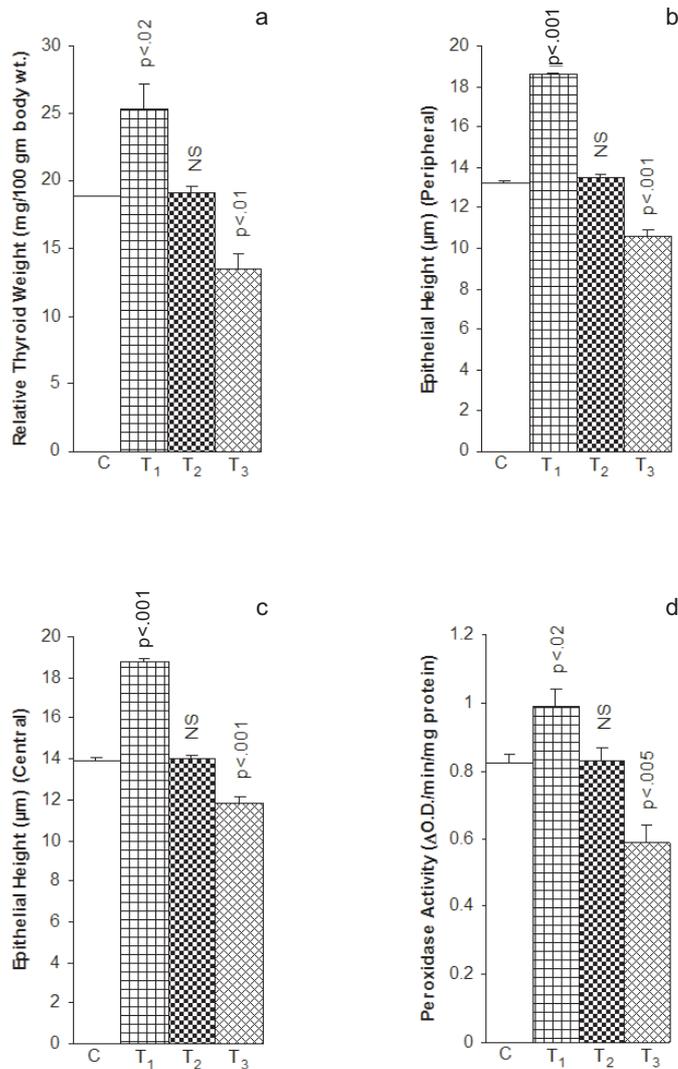
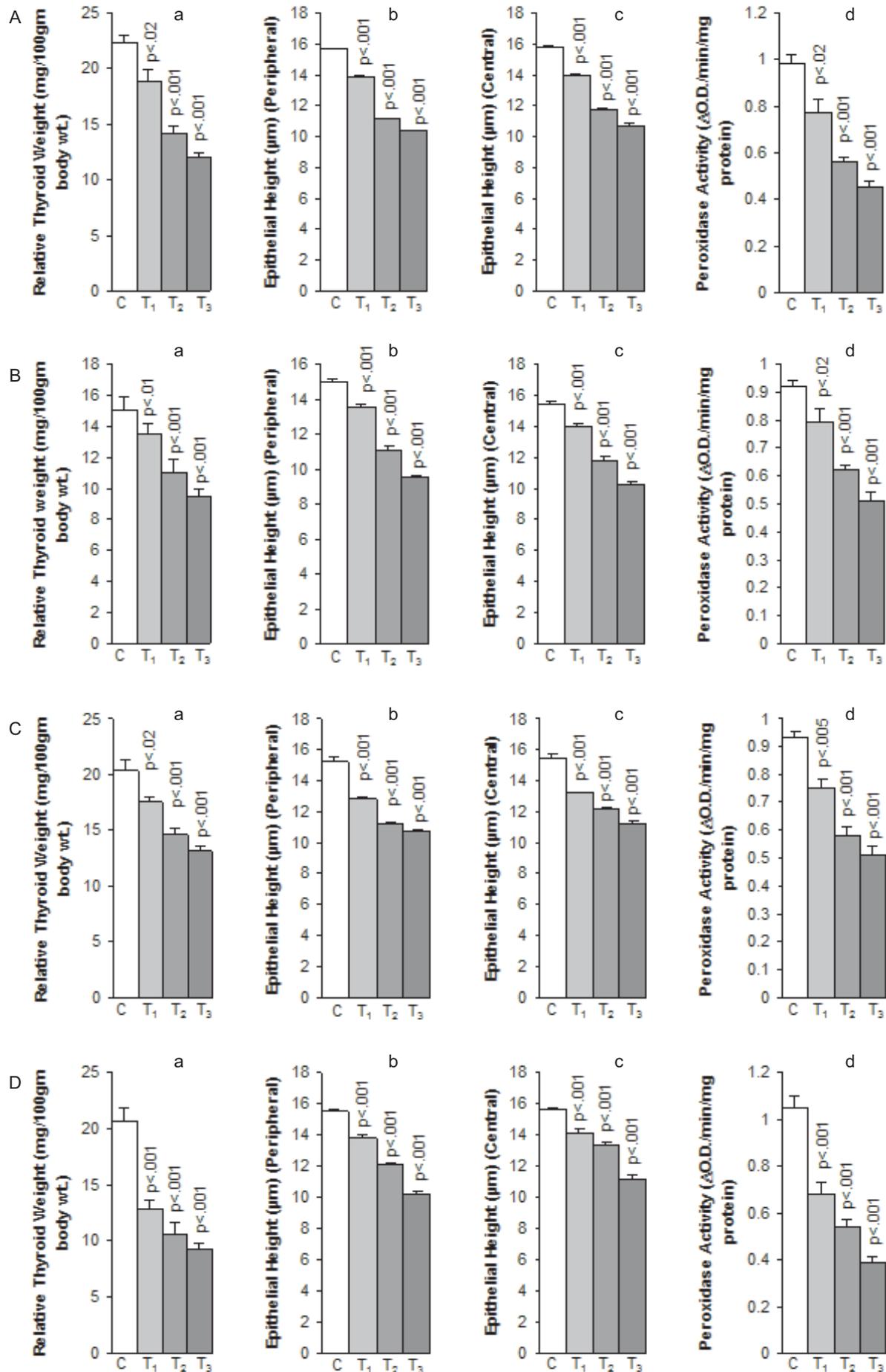


Fig. 1a-d: a – Histograms showing increased relative weight, b – increased thyro-follicular epithelial heights of the peripheral follicles and c – central follicles, and d – increased peroxidase activity of the thyroid gland after thyroxine treatment in low dose (T₁ : 25 $\mu\text{g}/100\text{ g}$ body weight daily for 10 days), without any significant change in these parameters in moderate dose (T₂ : 50 $\mu\text{g}/100\text{ g}$ body weight daily for 10 days, and decreased values of all the parameters (decreased relative thyroid weight, epithelial heights and thyroperoxidase activity) in higher dose (T₃ : 100 $\mu\text{g}/100\text{ g}$ body weight once daily for 10 days) in turtles. (ANOVA for all except medium 50 μg dose : P<0.01). (Level of significance by Students' *t*-test is shown in the figure).



Results

Control

Thyroid weight

The relative thyroid weight of the control animals is presented in Figs 1a, 2a of A, B, C & D.

Histology

The thyroid gland, encapsulated by a thin layer of connective tissue, consists of follicles of various shape and size. Each follicle is lined by a single layer of short columnar cells with heterogeneous colloid materials stored in the lumen (Fig. 3A). No significant difference in cell size or epithelial height was marked between the peripheral and central follicles (Figs 1b & c, 2b & c of A, B, C & D).

Thyroperoxidase

The enzyme activity of the control animals is presented in Figs 1d, 2d of A, B, C & D.

Treated

Thyroid weight

Thyroxine treatment significantly increased the relative weight of the thyroid gland only in lower dose (25 μg), failed to significantly alter the weight in moderate dose (50 μg) and decreased it significantly in higher dose (100 μg) (Fig. 1a). All other hormones (corticosterone, epinephrine, norepinephrine and insulin) decreased the relative weight of the thyroid gland of turtles (Figs 2a of A, B, C & D). The changes observed after the treatments were dose-dependent.

Histology

Thyroxine treatment increased the cell size from the short columnar to the columnar type with the appearance of heterogeneous vacuolated colloid materials in the follicular lumen of the thyroid in lower dose (25 μg) (Fig. 3B), failed to alter any perceptible change in moderate dose (50 μg) and decreased cell size from the short columnar to the cuboidal type with the homogeneous appearance of luminal colloid materials in higher dose (100 μg) (Fig. 3C). Simultaneously, thyro-follicular epithelial height in both the peripheral and central follicles was increased in low dose (25 μg), re-

mained unaltered in moderate dose (50 μg) and decreased in higher dose (100 μg) of thyroxine (Figs 1b & c). All other hormones (corticosterone, epinephrine, norepinephrine and insulin) decreased the cell size from short columnar to the cuboidal type in both the peripheral and central follicles of the gland. The colloid materials in the follicular lumen were homogeneous after the hormonal treatments. The epithelial height of the thyroid follicles was decreased in both the peripheral and central follicles of the gland (Figs 2b & c of A, B, C & D). These changes were observed in all the doses of the hormones administered but hormonal actions were dose-dependent (Figs 4A-D).

Thyroperoxidase

Thyroxine treatment increased peroxidase activity in low dose (25 μg), failed to alter the enzyme activity in moderate dose (50 μg) and decreased it in higher dose (100 μg) in turtles (Fig. 1d). Enzyme activity was decreased in all doses of all other hormones administered in turtles. The changes in peroxidase activity were dose-dependent (Figs 2d of A, B, C & D).

Discussion

Exogenous thyroxine has differential action on thyroid activity in soft-shelled turtles. Thyroxine in low dose (25 μg / 100 g body weight) stimulates thyroid activity by increasing the relative weight, follicular epithelial height and peroxidase activity of the thyroid gland, but the hormone is ineffective in moderate dose (50 μg / 100 g body weight), as there is no perceptible change marked in the values of these parameters. Whereas, thyroxine in higher dose (100 μg / 100 gm body weight) inhibits thyroid activity as evident from the reversed changes to those of the low dose of the hormone administered. Since thyroperoxidase is known to help in iodide transport, oxidation of inorganic iodide to organic iodine and iodination of tyrosil residues (thyroid hormone synthesis) (LARSEN *et al.* 2003), alteration of the enzyme activity might reflect changes in one or more steps of these events leading to an alteration in thyroid hormone synthesis in turtles. Thus, it appears that thyroxine has a biphasic action on thyroid activity in turtles because exogenous thyroxine stimulates thyroid activity initially in lower dose, but inhibits subsequently

Fig. 2. a – Histograms showing decreased relative weight, b and c – decreased epithelial heights of the peripheral (b) and central (c) follicles, and (d) – decreased peroxidase activity of the thyroid gland in all the doses of the hormones (A – corticosterone, B – epinephrine, C – norepinephrine and D – insulin) administered in turtles. (Group C : control : vehicle, groups T₁ : 25 μg , T₂ : 50 μg and T₃ : 100 μg each per 100 g body weight daily for 10 days). The changes were dose-dependent. (ANOVA for all, P<0.01). (Level of significance by Students' *t* test is shown in the figure).

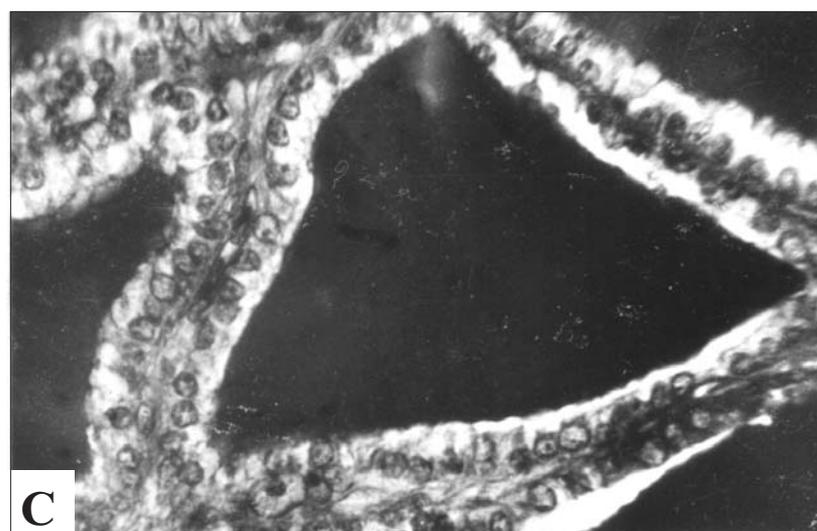
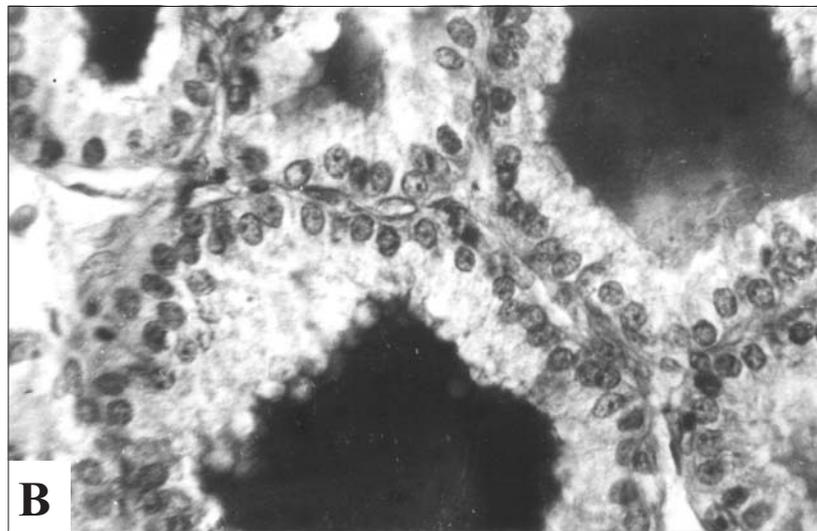
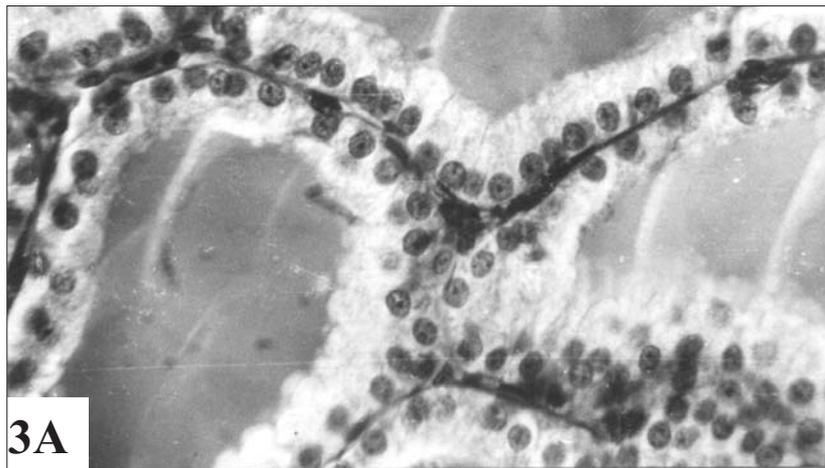


Fig. 3. A – A section through the thyroid gland of control turtle showing short columnar cells in the peripheral follicles with heterogeneous colloid materials found in the lumen. B – Thyroxine treatment in low dose ($25 \mu\text{g}/100 \text{ g}$ body weight, once daily for 10 days) showing increased cell size from the short columnar to the tall columnar type, with increased epithelial height and heterogeneous vacuolated colloid materials found in the thyro-follicular lumen of turtles. C – Thyroxine in higher dose ($100 \mu\text{g}/100 \text{ g}$ body weight once daily for 10 days) showing decreased cell size from the short columnar to the cuboidal type and decreased secretory activity with homogeneous colloids in the thyro-follicular lumen of turtles. Masson's trichrome stain. Photomicrographs, $\times 700$.

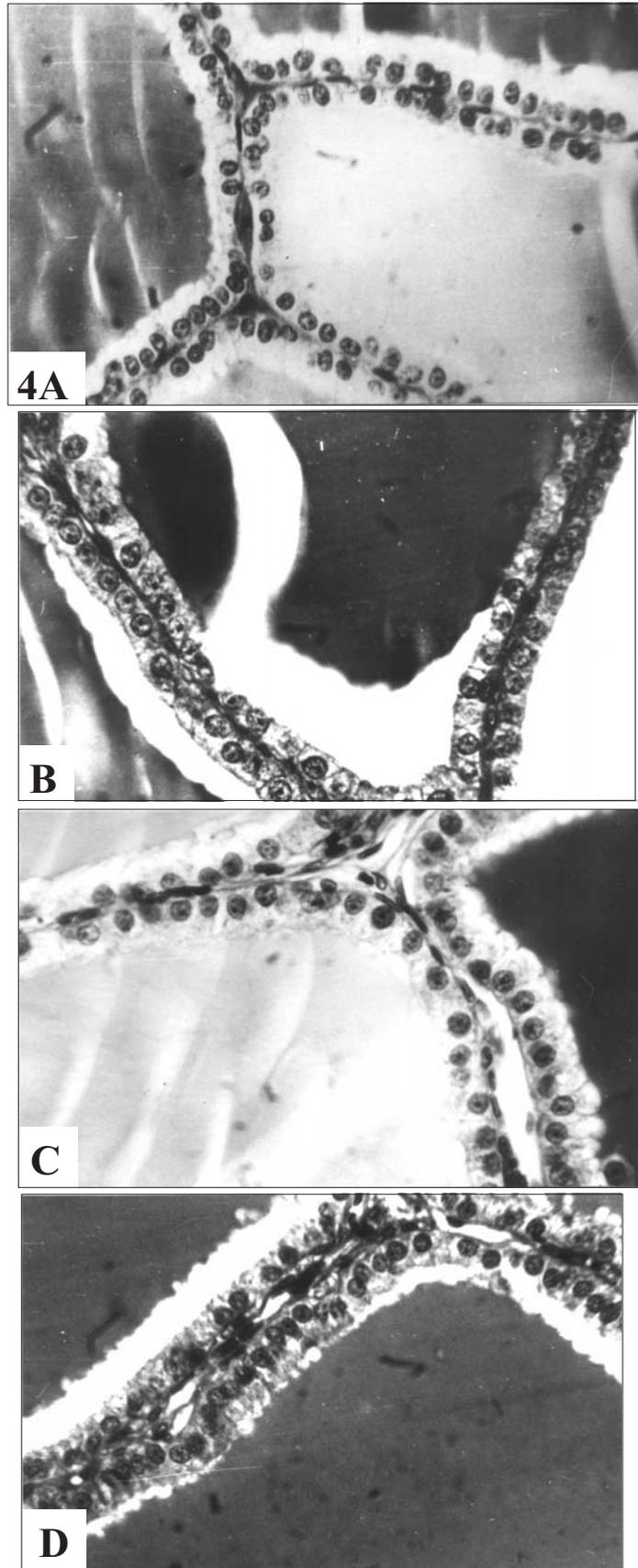


Fig. 4. Sections through the thyroid glands of turtles treated with the hormones (A – corticosterone, B – epinephrine, C – norepinephrine and D – insulin) administered in high dose ($100 \mu\text{g}/100 \text{ g}$ body weight daily for 10 days) showing decreased epithelial cell size from short columnar to the cuboidal type and decreased secretory materials with the appearance of the homogeneous colloids in the follicular lumen. Compare with the Fig. 3A of the untreated control. Masson's trichrome stain. Photomicrographs, $\times 700$.

with higher dose in turtles. Earlier authors have also observed suppression of thyroid activity by exogenous thyroxine in mammals (CSABA & NAGY 1986), birds and reptiles (CHIU *et al.* 1967) which is comparable to the present result of thyroidal inhibition following high dose of thyroxine, unlike thyroid stimulation in low dose in *Lissemys* turtles. It is known that low dose of T₃ / T₄ stimulates TSH release from the pituitary gland resulting in the stimulation of the thyroid gland, while a high dose reverses the effect (BARANOV *et al.* 1970; FRANCIS 1972). Endogenously low T₃ and T₄ levels make the thyrotroph cells more sensitive to TRF than that of higher T₃ and T₄ levels (cf. TURNER & BAGNARA 1976). In the slider turtle *Pseudemys scripta elegans*, it has been shown that pituitaries when incubated in different doses of LT₄ and T₃ cause reductions of both TSH secretion and the response to TRH, which indicates that thyroid hormones act at the level of the pituitary to regulate thyrotrophin secretion (DENVER & LICHT 1988). Nevertheless, in this study, a low dose of thyroxine (25 µg) stimulated thyroid activity presumably by enhancing TSH synthesis and/or release from the pituitary, or by increasing the sensitivity of thyrotroph cells to TRF. However, a high dose of thyroxine suppresses thyroid activity presumably through a reversed mechanism of stimulation (inhibition of TSH synthesis / release from the pituitary, or by decreasing the sensitivity of thyrotroph cells to TRF by negative feedback action). It is suggested that a low level of exogenous thyroxine may have stimulated TSH synthesis and/or release which in turn stimulated thyroid activity, whereas a high level of exogenous thyroxine might have inhibited pituitary TSH synthesis and/or release by a negative feedback mechanism in turtles.

Exogenous corticosterone inhibits thyroid activity by decreasing the relative thyroid weight, thyro-follicular epithelial height and thyroperoxidase activity in turtles. Thyroxine synthesis is probably suppressed because thyroperoxidase activity which is known to be related to hormone synthesis (LARSEN *et al.* 2003), is decreased after corticosterone treatment in turtles. Corticosterone also acts in a dose-dependent manner since the values of these parameters decreased with the increase in the dose of the hormone administered. Inhibitory effects of glucocorticoid, mineralocorticoid and ACTH on thyroid activity have also been reported in mammals (MALENDOWICZ & FILIPAC 1975), birds (MITCHELL *et al.* 1986; BUYSE *et al.* 1986) and fishes (REDDING *et al.* 1986). There are other reports that exogenous cortisone or ACTH suppresses thyroid activity by suppressing pituitary TSH or by reducing the pituitary stimulus to thyroid in mammals. Exogenous TSH elicits

thyroid response in the adrenocorticoid hormone-treated animals. Cortisol inhibits the accelerated release of thyroid hormones in TSH-treated dogs (ACKERMAN *et al.* 1961). ACTH or cortisone decreases the TSH-induced thyroidal uptake of ¹³¹I in the hypophysectomized rat. Cortisone also reduces the rate of radiiodine release from the thyroid gland. Nevertheless, in the present study in *Lissemys* turtles, whether corticosterone inhibits thyroid activity directly at the extra pituitary level (adrenal cortex) or via hypothalamo-hypophysial axis, needs to be confirmed.

Adrenomedullary epinephrine and norepinephrine also inhibit thyroid activity in turtles, since the relative thyroid weight, epithelial height as well as peroxidase activity decrease after the treatments. Thyroid hormone synthesis is probably also inhibited because thyroperoxidase which is known to help in thyroid hormone synthesis (LARSEN *et al.* 2003), is also inhibited after hormonal treatment in turtles. The actions of these hormones are also dose-dependent, since the degree of thyroid response (inhibition) increases with the increase in the dose of the hormone administered in turtles. The present findings corroborate earlier studies which also indicate inhibitory actions of adrenomedullary hormones on thyroid activity in rats and mice (TAKAMURA *et al.* 1982). Epinephrine and norepinephrine are known to act through different pathways. Epinephrine injection decreases ¹³¹I uptake by the thyroid in intact animals, but increases the uptake in the adrenalectomized animals which indicates that epinephrine acts directly or via the adrenal cortex. Epinephrine in higher dose causes acute suppression of plasma T₄ level (by inhibiting T₄ release from the thyroid) by acting as a local neurotransmitter involving the thyroid, hypophysis or hypothalamus (EALES *et al.* 1986). Norepinephrine suppresses TSH-stimulated T₄ release through modulation (decline) of α-adrenergic receptors (MAAYAN *et al.* 1981) without reducing the TSH-induced cyclic AMP level (TAKAMURA *et al.* 1982). Thus, in the current experiment, epinephrine inhibits thyroid activity directly or via adrenal cortex or acts as a neurotransmitter involving the hypothalamo-hypophysial axis. Norepinephrine exerts its inhibitory action involving α-adrenergic receptors – pituitary (TSH release and/or synthesis) or α-adrenergic receptors – hypothalamons (TRF) – hypophysial (TSH) axis in turtles, but such mechanisms of action of epinephrine and norepinephrine need to be confirmed.

Exogenous insulin, like other hormones (corticosterone, epinephrine and norepinephrine) inhibits thyroid activity of the soft-shelled turtles, since the relative weight of the thyroid gland, follicular epithelial height and thyroperoxidase activity are

decrease. Since thyroperoxidase is known to be involved in various steps of synthesis of thyroid hormones, decreased enzyme activity might also reflect the synthesis and/or release of thyroid hormones in insulin recipient turtles (LARSEN *et al.* 2003). The action of insulin on thyroid activity is also dose-dependent since the degree of response (inhibition) increases with the increase in dose of the hormone administered. The present findings in turtles corroborate those in birds (MITCHELL & RAJA 1986) which, unlike mammals, show stimulation of thyroid activity by exogenous insulin treatment (LEUNG *et al.* 1975; BLUM *et al.* 1977). Whether this discrepancy between reptiles including turtles (poikilotherms) and mammals (homeotherms), is related to different adaptations of poikilothermic and homeothermic vertebrates, species, sex and age of the animals, needs to be ascertained. Insulin administration induces sustained hypoglycemia which in turn suppresses thyroid activity since inadequate glucose delivery is known to inhibit pituitary TSH (ROJDMARK & NYGREN 1983; MITCHELL & RAJA 1986). Moreover, aside from adrenocortical involvement in stress (BENTLEY, 1998), insulin acting as a stress factor is known to elevate plasma catecholamines (KHALIL *et al.* 1986). Thus, in the present experiment, exogenous insulin may have exerted its action through sustained hypoglycemia resulting in TSH suppression which in turn inhibited thyroid activity (insulin-hypoglycemia-TSH-thyroid axis) in turtles. Otherwise, insulin, acting as a stress factor, might have increased the plasma catecholamine level which in turn inhibited thyroid activity. But catecholamines, particularly epinephrine, are well known hyperglycemic hormones (cf. BENTLEY 1998). Thus, insulin-induced hypoglycemia could inhibit thyroid activity presumably without involving adrenal catecholamines in turtles. Whether the precise mechanism of action of insulin on thyroid activity occurs via hypoglycemia and/or through catecholamine stimulation needs to be ascertained in turtles. Nevertheless, decreased thyroid activity following most of the hormones examined could be due to the pharmacological effects of the hormones administered, because the dose of the different hormones may be higher than that of the physiological dose required for thyroid responses (stimulation?) in turtles.

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