Adrenocortical involvement during diverse stress in soft-shelled turtle

Lissemys p. punctata Bonnoterre

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Adrenocortical responses to diverse stressful situations (dehydration, formaldehyde treatment and salt loading) were studied in the adult female soft-shelled turtle, *Lissemys p. punctata*. Dehydration, formaldehyde treatment (formalin, 1%: 0.1 ml/100 g body weight daily) or salt loading (NaCl, 1%: 0.1 ml/100 g body weight daily) treatments consecutively for 7 days caused hypertrophy of the adrenocortical cells with their nuclear diameter increased, and depletions of adrenal cholesterol and ascorbic acid concentrations followed by decreased acid phosphatase and alkaline phosphatase activities in turtles. Corticosterone levels were elevated in both the adrenal gland and serum of turtles after dehydration and formalin stress, but the hormone level ramined unaltered after salt loading in turtles. The results suggest active involvement of adrenal cortex in stress for homeostasis in *Lissemys* turtles.

**Keywords:** Adrenal cortex, Stress, Histology, Cholesterol, Ascorbic acid, Phosphatases, Corticosterone, Turtle

Adrenocortical participation during diverse stress (aggression, cold exposure, hunger, pain, surgery, burn, formaldehyde, histamine shock, insulin shock, etc.) is fairly well-known in mammals. Though surgery is one of the most potent activators of the hypothalamo-pituitary-adrenal axis, the maximum ACTH secretion occurs during reversal of anaesthesia, exturbation and immediate post-operative recovery period. Acute psychological stress also enhances ACTH and cortisol secretions\(^1\,\,\,^2\). Diverse stress such as dehydration, saline loading, formalin shock, cold-wet-immobilization, etc. can cause adrenal cell or nuclear hypertrophy, hyperplasia, depletions of ascorbic acid, cholesterol and sudanophilic lipids, or fall in plasma corticosterone level in several avian species. Even formalin stress can induce adrenocortical hypertrophy and hyperplasia in the regressed adrenal of hypophysectomized pigeons with lesion in median eminence\(^3\,\,\,^5\).

Although reptiles are successful in many habitats, little is known about the role of adrenal cortex in mediating environmental adaptations. Plasma corticosterone level is elevated following salt loading or confinement in the desert lizards, *Aneilobolurus ornatus* and *Dipsosaurus dorsalis* and during bleeding stress in *Caiman* crocodiles. Some information is also available on the adrenocortical responses to stress in turtle. Adrenal corticosterone concentration is depleted after acute hyperosmotic stress in *Lissemys* turtles\(^6\), but plasma corticosterone level is elevated during mass nesting stress in sea turtles *Caretta caretta*\(^7\) and *Lepidochelys olivacea*\(^8\). High-density nesting turtles elaborate more plasma corticosterone (plasma B) than turtles in low-density sectors, though the magnitude of this increase is small, without any change in plasma B during prolonged successful oviposition in green turtles (*Chelonia mydas*) of Raine island of Northern Great Barrier Reef\(^9\). Plasma corticosterone level increases to 16-fold during hyperthermia or capture stress induced by rising of body temperature from 28.2⁰C to 40.7⁰C in less than 6 hr despite the lethal stressor in breeding sea-turtles (*Chelonia mydas*), but the absolute increase in plasma B was small and much less than expected, despite the lethal stress\(^10\). These evidences further indicate that reduced adrenocortical function operates in breeding green turtles in presence of most pervasive environmental stressors\(^10\). There is practically no information on adrenocortical function during stress in fresh water turtles as yet. In the current article, this problem has been examined in *Lissemys* turtles. Stress
experiments were conducted in January because adrenocortical activity was low in this month of the year as evident from seasonal adrenocortical study of *Lissamys* turtles.

**Materials and Methods**

Adult female soft-shelled turtles, *Lissamys punctata punctata* (20, body weight ranging from 1150±50g) were collected from natural populations near Calcutta. They were kept in the aquaria (150 cm × 90 cm × 90 cm) in controlled temperature (25°C) and photoperiod (11L : 13D) with food (tubifex and shrimps) accessible *ad libitum* for 5 days prior to study for acclimatization in the laboratory conditions. Animals were equally divided into four groups (I, II, III and IV) of 5 each in separate aquarium. Group I served as control. Groups II were kept in complete deprivation of water for dehydration experiment; group III received formalin (Formaldehyde, E. Merck, 1%) injection, im at a dose of 0.1 ml/100 g body weight daily at 1000 hrs and group IV animals were injected with salt solution (NaCl, Sigma, U.S.A.: 1% aqueous) in a similar dose, mode and time of treatment. Control animals received distilled water in similar dose. All the experiments continued consecutively for 7 days. Animals were killed by decapitation at a particular time of the day (at 1000 hrs) to avoid effects due to diurnal rhythm. Both the adrenals were quickly dissected out and left adrenal of each specimen was fixed in Bouin’s fluid for histological study. Following routine microtomy 5 μm thick paraffin sections were prepared and stained by Masson’s trichrome technique. Adrenocortical cell nuclear diameter (mean of long and short axes in μμ) was measured by an ocular micrometer. Nuclear diameter was studied following the method of Abercrombie. One hundred nuclei each from the subcapsular and central zones of the adrenal gland were considered from 10 widely separated random sections of the adrenal gland of each specimen.

Total ascorbic acid, cholesterol, acid phosphatase and alkaline phosphatase concentrations were estimated from the remaining right adrenal glands. All colorimetric samples were measured on a PERKIN-ELMER Spectrophotometer (550 S, West Germany). Additionally, adrenal (right) and serum corticosterone concentrations were also studied. Blood was drawn from the heart in heparinized syringe, and serum was collected and assayed for corticosterone. Corticosterone was extracted from serum and adrenal gland, and was measured by spectrofluorometer (HITACHI-Model 650-10M) following the method of Glick *et al.* All data were analyzed by one way ANOVA for ascertaining the statistical significance of the stress experiments.

**Results**

**Control**

Adrenal glands consist mostly of cortical strands intermingled with chromaffin elements. Gross histological structure of the adrenal gland of soft-shelled turtles has been described earlier (Fig. 1).

**Treated**

**I. Dehydration**

**Histology**

Adrenocortical strands became broader with conspicuous cell outline and hypertrophied cells. Cortical cell nuclei became prominent and their

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Figs. 1, 2.—Transverse section of adrenal gland of turtle showing 1: smaller adrenocortical cells (AC) and their nuclei (N) in the control turtle and 2: hypertrophied adrenocortical cells (HAC) and nuclei (N) with decreased nuclear population per microscopic field after dehydration stress. Masson’s Trichrome stain. (x 400).
diameters were increased (Figs 2 and 3) (changes in subcapsular nuclei: +16.36%, central nuclei: +20.89%). The findings were statistically significant $P < 0.05$. Nuclear population per microscopic field was decreased (25/microscopic field) compared to that of control (45/microscopic field).

**Biochemical changes**

a) Cholesterol and ascorbic acid: The concentrations were significantly depleted 7 days after dehydration (Fig. 3). (changes in cholesterol: free: -64.8%, esterified: -72.68% and in ascorbic acid: -40.35%).

b) Acid and alkaline phosphatases: Activities of both the phosphatases were increased significantly after dehydration (Fig. 3). (changes in acid phosphatase: +113.08%, alkaline phosphatase: +150.79%). All the biochemical findings were statistically significant ($P < 0.005$).

c) Corticosterone: Adrenal and serum concentrations were significantly increased ($P < 0.01$) after dehydration stress in turtles (Fig. 4). (changes in the gland: +52.94% and serum: +29.41%).

### II. Formaldehyde treatment

#### Histology

Cortical strands were enlarged with hypertrophied cells and their nuclear diameters were increased (Fig. 3) as compared to those of control and the findings were statistically significant ($P < 0.05$). (changes in subcapsular nuclei: +18.18% and central nuclei: +17.85%).

**Biochemical changes**

a) Cholesterol and ascorbic acid : The levels were significantly depleted after formaldehyde treatment (Fig. 3). (changes in cholesterol: free: -68%, esterified: -66.96% and total: -67.32%, and in ascorbic acid: -39.58%).

b) Acid and alkaline phosphatases : The activities of acid and alkaline phosphatases were increased after formaldehyde treatment (Fig. 3). (changes in acid phosphatase: +90.50% and alkaline phosphatase: +136.98%). All the changes in biochemical components were statistically significant ($P < 0.005$).

c) Corticosterone : Adrenal and serum levels were increased significantly ($P < 0.01$) following formalin stress in turtles (Fig. 4). (changes in the gland: +29.41% and serum: +14.92%).

### III. Salt loading

#### Histology

Adrenocortical strands were enlarged with hypertrophied cells. Nuclear diameter was also increased significantly ($P < 0.05$, Fig. 3). (changes in subcapsular nuclei: +22.72% and central nuclei: +19.64%).

**Biochemical changes**

a) Cholesterol and ascorbic acid : The concentrations were depleted after salt loading (Fig. 3). (changes in cholesterol: free: -58.80%, esterified: -20.92%, total: -34.37% and in ascorbic acid: -43.84%).
b) Acid and alkaline phosphatases: Activities of both the enzymes were significantly increased (Fig. 3). (changes in acid phosphatase: + 52.51% and alkaline phosphatase: + 80.95%). The changes in cholesterol, ascorbic acid and phosphatase were statistically significant ($P < 0.005$).

c) Corticosterone: Adrenal and serum corticosterone levels were not significantly altered after salt loading in turtles (Fig. 4). (changes in the gland: + 0%, and in serum: + 4.47%).

Discussion

Poikilothermic animals, like homeotherms, can also respond to diverse stressful situations. Stressors like dehydration, formaldehyde and salt loading when applied continuously for 7 days, caused significant stimulation of adrenocortical activity as evident from cellular hypertrophy with increased nuclear diameter and elevations of acid phosphatase and alkaline phosphatase activities accompanied by depletions of cholesterol and ascorbic acid concentrations in turtles. It is known that increased nuclear diameter and phosphatase (acid and alkaline) activities or depletions of cholesterol and ascorbic acid levels\(^{13}\) of the adrenal gland reflect adrenocortical hyperactivity including corticoidogenesis. Parallel changes in these parameters have also been reported after thermal stress (hyperthermia) in turtles\(^{14}\). Moreover, in the present experiment corticosterone levels were also elevated in the adrenal gland and serum following dehydration and formalin stress in turtles; it is likely that both the synthesis and release of corticosterone were stimulated by dehydration and formalin stressors. Romero\(^{8}\) reported that stress causes glucocorticoid release through activation of hypothalmo-pituitary-adrenal (HPA) axis that induces a variety of behavioural and physiological changes presumably to help the animal to respond appropriately to the situation. In the present study a rise in corticosterone (glucocorticoid: GC) level was recorded in both the adrenal gland and blood following dehydration and formalin stresses in turtles, and this finding not only confirms that stress induces corticosterone (GC) release but also stimulates corticosterone synthesis in Lissemys turtles. Stress-induced corticosterone synthesis and release into circulation may presumably help to adapt the animal to the stressful situation for survival through stress-energetics-concept. Earlier studies have also reported similar results of the rise in adrenal corticosterone level after dehydration, formalin plus cold-wet-immobilization (7 days) or hyperosmotic (acute for \(1/2\) hr to 2 hr) stress in birds\(^{15}\). It is interesting to note that in the present experiment although salt loading stress caused adrenocortical stimulation, corticosterone levels in the adrenal gland and blood were not significantly altered after hyperosmotic stress in turtles. In an earlier study it has been reported that acute hyperosmotic stress for \(1/2\) to 2 hr decreased corticosterone concentration of the adrenal gland of turtles\(^{6}\). It may so happen that hyperosmotic stress either stimulated the release of corticosterone into circulation resulting in the loss of corticosterone in the adrenal gland. Otherwise, it stimulated the synthesis of corticosterone which in turn have been converted into

![fig-4](https://example.com/fig-4.png)
aldosterone, since corticosterone is known to be the precursor of aldosterone\(^8\) that eventually cuses loss of corticosterone in the adrenal gland. Such a possibility cannot be ignored because aldosterone is known to regulate mineral metabolism. Moreover, involvement of angiotensin along with aldosterone during osmotic stress is quite plausible since both are known to regulate ionic balance in vertebrates\(^8\), but it has to be confirmed. Dehydration and formalin stress or hyperthermia have been reported to stimulate adrenomedullary hormonal level (epinephrine and norepinephrine) and consequently blood sugar level in the same species of *Lissennys* turtles\(^17\). Nevertheless, the present findings in turtles corroborate with those of earlier works in lizards and turtles, birds and mammals\(^3,5\) during hyperthermia\(^3,14\).

In the present turtle species, there is a report of positive correlation between the seasonal adrenocortical cycle and the ovarian cycle, since the adrenocortical activity is stimulated during peak ovarian activity\(^5,9\). Additionally, exogenous estradiol also caused stimulation of adrenocortical function in *Lissennys* turtles\(^20\). Such a positive association between corticosterone and reproduction has also been reported to exist as evident from a number of earlier studies in reptiles and amphibians\(^2,5\), but this finding contradicts the generalization that stress inhibits reproduction. Moore and Jessop\(^9\) have suggested that moderate stress facilitates reproduction while extreme stress situation inhibits it. In the latter case, hypothalamic-pituitary-adrenocortical (HPA) axis is extremely activated which in turn elevates extremely high level of corticosterone that inhibits reproduction. Further, modulation of HPA depends on ecologically based variables, like variability in length of breeding season and lifetime reproductive opportunities. Corticosterone level has also been reported to be stimulated during reproductive stress, but not significantly in *Lepidochelys olivacea*\(^6\). It is suggested that the down regulation or desensitization of acute adrenocortical response occurs for adaptive trade-off mechanism for optimizing current reproductive success by preventing physical and behavioural disturbance interfering with reproduction\(^22\), but the exact mechanism by which adrenocortical corticosterone stimulation following high population density is prevented in nesting turtles remains unclear.

It is interesting to note that although dehydration, formalin or salt loading stress can stimulate adrenocortical activity, the degree of response to these stressors appears to be different with different stressors in turtles, since dehydration and formaldehyde stressors caused greater stimulation of adrenocortical activity than that induced by salt loading, thereby indicating that the former stressors are more potent than the latter in the present study. It is known that the stress is mediated by CNS-hypothalamo-hypophysial-adrenocortical axis in homothermic animals. Stress exerts its effects through unknown central pathways that stimulate the hypothalamus to release multiple ACTH secretagogues, CRH and AVP being the most important\(^23,16\). Thus, such a mechanism may explain the current adrenocortical stimulation following stress in *Lissennys* turtles. In essence, all the stressors stimulate adrenocortical activity including corticosterone to maintain homeostasis under conditions of stress in turtles like homeothermic vertebrates.

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