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Radioimmunoassay of cortisol in blood of buffaloes *(Bubalus bubalis)* during the oestrous cycle[1]

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Summary. Changes in the concentration of cortisol were measured by radioimmunoassay in the blood plasma of buffaloes *(Bubalus bubalis).* There were minor fluctuations in the level during the oestrous cycle, but the differences between the days were not significant. The study revealed that cortisol under normal conditions does not appear to be involved in the regulation of the cycle.

The response of domestic animals to various physiological, environmental and management-related stresses is characterized by activation of the hypothalamic pituitary adrenal axis. Determination of circulating cortisol is a useful parameter for assessing adrenocortical activity. The radioimmunoassay of cortisol in human blood plasma has been reported[3-5], but no attempt has been made to quantify this hormone in the blood plasma of buffaloes. The present study deals with the radioimmunoassay of cortisol in the blood plasma of buffaloes during the oestrous cycle.

Materials and methods. Animals and blood collection: 6 Murrah buffaloes with normal cycles were selected from the Institute herd, and blood samples were collected on alternate days between 10.00-12.00 h from the day of oestrus to the next oestrus. Blood was collected rapidly in tubes packed in ice and centrifuged at 4 °C; plasma was stored at −20 °C pending hormonal analysis. Maximum care was taken while collecting blood to avoid any possible stress which might influence the hormone level.

Radioimmunoassay: Duplicate (1 ml) plasma samples were vortexed vigorously for 1 min with 10 ml distilled dichloromethane. Following brief centrifugation, the upper aqueous plasma layer was removed and the lower organic layer was transferred and evaporated to dryness under nitrogen in a 40 °C water bath. The residue was dissolved in 200 µl of 0.1 M phosphate buffered saline, by keeping the tubes at 45 °C for 15 min and vortexing for 30 sec. The remaining procedure was same as reported earlier from this laboratory by Arora et al.[6]. The validity of the assay was determined by adding known amounts of cortisol in charcoal-treated (hormone free) plasma. Recoveries of 0.5, 1.0, 2.0 and 4.0 ng of cortisol added to 1 ml of charcoal-treated plasma were 0.54±0.08, 1.12±0.07, 2.08±0.03 and 4.08±0.08 (mean±SEM) respectively. The sensitivity of the assay was 0.075 ng. The coefficients of intra- and inter-assay variations were 11.8 and 13.9%. The antiserum cross-reacted with 30% deoxycortisone and 4.1% corticosterone; cross-reaction was very low with other steroids.

Results and discussion. The changes in the concentration of cortisol during the oestrous cycle are shown in table 1. The level on the day of oestrus was 14.00±0.53 ng/ml, and fluctuated between 8.36 and 15.03 ng/ml during the oestrous cycle.

Our observations revealed minor fluctuations in the levels of plasma cortisol during the oestrous cycle, and the differences between the days were not significant (table 2). This pattern for cortisol level during the oestrous cycle was similar to that observed in the ewe[7] and in dairy cows[8-11]. On the other hand, Swanson et al.[12] and Gimenez et al.[13], for heifers, and Bhattacharya et al.[14], for goats, have described a higher level of glucocorticoids at oestrus compared with the level observed during the rest of the cycle. Sprague et al.[15] showed that adrenal release of corticoids under stress conditions may influence the oestrous cycle. Since in our study maximum care was taken to avoid stress while collecting blood, differences in cortisol levels between the days of the cycle cannot be attributed to stress. This implies that cortisol under normal conditions does not

Table 1. Cortisol concentration (ng/ml) in blood plasma of cycling buffaloes

Days	No. of observations	Concentration ± SE
0	6	14.00±0.53
1	6	9.70±0.52
3	5	9.97±1.98
5	6	8.36±1.47
7	6	10.53±1.71
9	6	11.28±1.65
11	6	11.88±2.66
13	6	11.34±2.44
15	5	12.14±1.49
17	6	11.44±1.51
19	5	15.03±2.01
−4	6	10.87±1.12
−2	5	11.70±1.13
0	4	14.37±0.59

Table 2. ANOVA

Source of variation	df	Mean squares	F
Between days	13	20.373	1.448*
Within days (error)	67	14.065	

* Non-significant.

appear to be involved in the regulation of the oestrous cycle. The estimation of corticoid levels may be useful to determine whether stress has resulted from the handling of the experimental animals and sample collection.

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Ontogenic development of the renal ornithine decarboxylase response to testosterone

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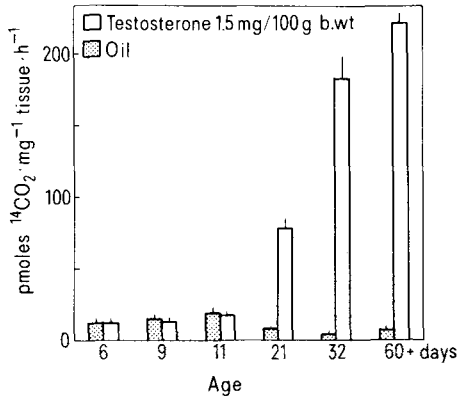
Summary. In neonatal mice, renal ornithine decarboxylase was not altered by testosterone injection, in contrast to adult mice in which the enzyme was greatly elevated following treatment with testosterone.

Ornithine decarboxylase (ODC, EC 4.1.1.17) is the rate-limiting enzyme in the biosynthesis of the polyamines, a group of aliphatic cations frequently associated with cellular profileration[1]. Numerous studies have demonstrated that ODC activity and polyamine concentrations are highest during rapid growth of regenerating tissues and during embryonic development of various animals[1]. In rat embryos ODC activity peaks on fetal days 12–15 and then declines to very low levels[2]. Intracellular polyamine concentrations follow this pattern closely.

ODC activity is hormonally sensitive and responds quickly, usually within 4 h to either peptide or steroid hormonal stimulation[3]. In the murine kidney testosterone propionate (TP) elicits an increase in ODC activity greatly exceeding that for other renotropic agents[4]. Ontogenic patterns of response to hormonal stimulation are exhibited by several renal enzymes[5], but such a pattern has not been demonstrated for ODC. We have therefore examined the renal ODC response to TP injections in neonatal and adult mice. ODC activity, as measured by in vitro production of $^{14}CO_2$ from DL-(1-^{14}C)-ornithine[4], was increased 2000% in the kidneys of adult male Nya: NYLAR mice 15 h after a s.c. injection of TP (1.5 mg/100 g b. wt) suspended in sesame oil. At postnatal day 6, by contrast, there was no renal response to TP injection when compared with oil injected controls (fig.). The neonatal response to TP increased slightly during the suckling period but was not maximal until after puberty. Control ODC activity in the kidney peaked at 11 days and then declined to adult levels at weaning – a pattern comparable to that of other enzymes[6]. Rajerison et al. reported a similar developmental pattern for renal response to vasopressin[5]; however, the response to vasopressin increased progressively, and the kidney never failed to respond to the hormonal stimulus. The authors attributed this phenomenon to an ontogenic development of vasopressin binding sites in the young rats[5]. Our results suggest that the renotropic effects of TP may be similarly related to kidney maturational processes. These could involve receptor changes, as in the vasopressin study, or a temporal deficit of intracellular effectors, such as cAMP[7]. Future studies are planned to clarify this relationship.



Effect of s.c. injection of testosterone propionate on renal ODC. Values are mean ± SEM for 8 mice in each group.

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