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CHANGES IN SERUM CONSTITUENTS OF AQUATIC FROG, *HOPLOBATRACHUS TIGERINUS* AND TERRESTRIAL TOAD, *DUTTAPHRYNUS MELANOSTICTUS*

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ABSTRACT

Determination of serum glucose, protein, cholesterol, urea, sodium, potassium and calcium were made in monthly sample of blood collected from male and female frogs and toads from tropical Western Ghats, India over a period of two years annual cycle. Serum glucose was significantly higher in frogs, whereas protein, cholesterol, urea, potassium and calcium concentration assumed higher in toads. However, sodium concentration did not alter throughout the study between the two species.Serum protein, cholesterol, potassium and calcium concentration were significantly higher during monsoon, whereas glucose was decreased. However, urea was rose to maximum during post monsoon period.

Keywords: Habitat, protein, season, serum, species.

INTRODUCTION

The studies on cyclic seasonal changes in amphibian blood biochemistry have been of considerable interest to herpetologists and zoologists, the information hitherto available, is by and large based on studies conducted on non-tropical species, either in their normal condition. Therefore, the investigation carried out thus for have been found on tropical species during their breeding period is not yet studied.

The objectives of the present study were to determine serum protein, cholesterol, glucose, urea, sodium, potassium and calcium in aquatic/semi aquatic frogs and terrestrial toads during the period of breeding and non-breeding over a period of two consecutive years of annual cycle pattern.

MATERIALS AND METHODS

Live and healthy frogs and toads of both sexes with a mean body weight of 89+1.6g and 78+0.9g with a range of 60 to 120g were collected from tropical Western Ghats regions of South Kannada District, India twice (the first and third week) every month for 24 consecutive months.

The animals were anaesthetised with diethyl ether. Blood was collected directly from the heart using 2.0 ml glass syringe fitted with No.21 glass needle. Approximately 1.5 ml of blood was drawn and then transferred to a graduated centrifuge tube containing plastic serum separator (this promotes clear separation because the separator forms an impermeable

stopper on top of the deposit of fibrin and blood cells upon centrifugation). It was allowed to clot for about 30 minutes at room temperature and then centrifuged for exactly 10 minutes at 2500 rpm in a RBC centrifuge. The serum was separated using a Pasteur pipette, transferred to a small test tube and frozen prior to biochemical analyses. The Serum glucose was estimated by Ortho-toluidine method (Henry *et al.*, 1974). Serum protein was measured by the method of Lowry *et al.* (1951) using BSA (Bovine Serum Albumin) as a standard. Cholesterol in the blood serum was estimated by Zak's method (Varley, 1976). Urea concentration in serum was assayed by diacetylmonoxime method (Glowenlock, 1988) Concentration of sodium and potassium in blood serum was determined by the emission flame photometry (Oser, 1976). Calcium content of blood serum was assayed by O-cresolphthaleincomplexone method of Baginski *et al.* (1973) using a standard kit provided by Reddy's laboratories, Hyderabad, India.

Statistical Analysis: Mean and standard of mean (SEM) of each parameter were computed using data from at least five males and females of frogs and toads in a given month. As there was no significant difference in mean values of each parameter of males and females of frogs collected in first week and those with third week, the data were pooled and expressed as mean values for a given month. A similar procedure was followed for toads. Data on each parameter of males and females of a given species for a given month were pooled to obtain month-wise mean SEM for the purpose of finding out significant differenceif any between two species studied. The data were analysed using one-way and two-way analysis variance (ANOVA) with season vs. species as factors. Further comparison of significant factors was made using multiple comparison tests (Duncan's multiple range test). The mean value of each parameter for different months in a given season waspooled to find out the percentage of variation. The difference in all the cases was judged as significant if $p<0.01$.

RESULTS

Table 1 shows serum glucose level was significantly higher in frogs than in toad throughout the year except in July and October the differences was not significant. On the other hand serum protein, cholesterol, urea, potassium and calcium reverse was true.

In both species serum glucose and urea had decreased during pre-monsoon to monsoon and increased in post-monsoon, whereas protein, cholesterol, potassium and calcium had tends to reverse and these changes were highly significant ($p<0.01$) (Table 1) However, sodium remains constant throughout the season in both species.

In both species of either sex serum glucose changes significantly with respect to season. A sex difference was recorded round the year resulting decidedly higher glucose in male Table 1). There was a decrease in monsoon and increase towards post monsoon. However, there was no significant change from post monsoon to pre monsoon. The seasonal changes in protein, cholesterol, potassium and calcium are given in Table 1. The observed differences, as treated by ANOVA are highly significant with seasons. Sex difference was significantly more pronounced during monsoon with females having greater values in their blood and are increased significantly in monsoon and decreased in post-monsoon and subsequent months. Seasonal changes in urea were given in Table.1. The observed changes, as treated by ANOVA, are highly significant seasonally and in particular months resulting decidedly higher urea in females round the year. In frogs urea decreased from pre-monsoon to monsoon and increased in post-monsoon and a second decrease in pre-monsoon. On the contrary, it increased in post-monsoon, decreased in pre-monsoon and increased in monsoon toads. No significant changes in Sodium with respect to season either with species or sex throughout the year.

DISCUSSION

The study reveals Serum Glucose was higher in frogs than toads throughout the twoyear period, as the two species differ mainly in their habitat ecology and feeding habit. The similar observation reported in *R. temporaria* and *Bufo bufo* (Hermansen and Jorgensen, 1969 and Harri, 1975). The results indicate that there is an apparent relationship between serum component and habitat of the animals. It is known that in amphibian blood glucose were much lower than those of homeotherms. In both species serum glucose fluctuates between 10-50 mg/dl with mean valuesof (22.0 mg/dl). The significantly

higher glucose in frogs than toads reflects in the former glucose is more important as energy source perhaps to cope with their very active life. On the contrary, protein, urea, potassium and calcium were higher in the latter than the former. The higher protein in toads could be due to nutritional factors, as the relatively protein rich food of the latter comprised of insects and worms. Higher urea level in toads suggests greater dehydration stress in a terrestrial habitat compared to aquatic medium of frogs. Relatively dry microhabitat of toads perhaps gives rise to increased level of serum potassium and calcium. The inter-specific difference also reported in reptiles. For example sodium and potassium are higher in terrestrial turtles than in aquatic forms (Palomoque *et al*., 1977). Similarly Lykakis (1971) and Musquera *et al*. (1976) reported higher protein in land turtles than in aquatic ones.

In both species serum components except sodium showed significant seasonal fluctuation through two year cycle whichis more pronounced during breeding season (monsoon). In both sex of either species there is significant decrease in glucose (62-28%). Conversely there is an increase in protein, potassium, urea (7- 135%) and calcium (10 to 12%) in females during monsoon. The frogs and toads breed during the months of June to September (monsoon) and in nature adult frogs and toads spawns in June and July months.

The major metabolite, serum glucose in both sexes of either species there is significant decrease from pre monsoon to monsoon (62 to 28%). It may be due to increased metabolic rate, conversely there is a reduction in carbohydrate. Therefore, one may infer that in monsoon there might be an increased tissue consumption of adrenal of the hormone from circulation. In the pre mating period however, a higher rate of secretion could be explain the maximum concentration of this metabolite. Decreased plasma adrenaline in mating season and progressive increase towards the end of summer have been reported for *Bufo arenarum* (Donoso and Segura, 1965) whereas, blood glucose increased during breeding season in spring and remains so during summer in frog *Rana pipiens* (Mizell, 1965) and higher plasma glucose in July and December was reported in Giant swamp frog, *Dicroglossus occipitalis* (Kuhn *et al*., 1987).

In both species protein was significantly increased from pre monsoon to monsoon (28 to 108%). It is indicative of increase in protein reserves. The further decrease in post monsoon may suggest complete depletion of protein reserves. In the turtle, *Chrysemys picta* serum protein was set a maximum in spring and falls in winter and summer where feeding ceases (Masat and Musachia, 1966). Similarly there is a significant increase in serum cholesterol from pre monsoon to monsoon (55 to 135%). The higher level recorded in monsoon could be indicative of increased production of sex steroids, as evidenced by the active state of testis and well developed ovaries in preparation for breeding, since cholesterol is a known precursor of sex steroids hormone. There is evidence that a similar rise in plasma cholesterol occurs in amphibians prior to ovulation (Follett and Redshaw, 1968).

There is limited information available at present pertaining to significance of urea in amphibian blood. The study on urea has a profound impact on the amphibian physiology and excretion. The study shows marked fluctuation in serum urea. The significant difference noticed between the high level in the frog and toad obtained in post monsoon and low level in monsoon period may be attributed to increased protein catabolism. Restricted feeding and low food availability in post monsoon might also have contributed to the increased urea since prolonged fasting has been shown to increase plasma urea in the salamander as a direct consequence of increased catabolism of tissue protein (Hervant *et al*., 2001). On the contrary, low urea during monsoon was probably indicative of the fact that protein was stored as energy fuel. The significantly higher urea in post monsoon suggests greater dehydration. In frogs and toads the physiological processes such as reproduction with their high energy demands, seems to have a great impact on the energy reserves in the body, including serum protein.

As per the study it may be predicted that in frogs and toads, serum calcium shows significant changes, the only exception is that in males of both species it did not varies significantly. In females of both species it was increased significantly (10-12%) from pre monsoon to monsoon and decreased in post monsoon. The significantly higher calcium in monsoon, since amphibian oviduct is able to store large amount

of calcium for egg shell formation, it must derive it from circulating blood. It may therefore conclude that the higher calcium in early July month (breeding period) was the result of calcium being mobilised to the uterus for egg shell formation. The sources for calcium for egg shell formation in amphibians are the diet. Hence, the species on their breeding ground could have obtained calcium that was readily available in the diet.

Total serum potassium in both species showed little variation outside the breeding season. During the months of October to January and February to May there were no significant differences. However, at the onset of vitellogenesis in June to August (monsoon) there was a significant rise (7-135%) and then from October to January (post-monsoon) after ovulation completed levels rapidly dropped to non-breeding levels (43-22%). There was highly significant correlation between body weight and serum potassium during breeding period. There were very few studies on reproductive cycles on amphibians and reptiles reported changes in serum potassium. However, plasma potassium is relatively constant throughout the year whereas plasma calcium is maximal in winter in frog, *R. pipiens* (Robertson, 1978). Similarly, plasma potassium did not alter during breeding season in female cobra, *Naja naja* (Lance, 1976). In male and female frogs there was a significant increase in serum sodium during monsoon to post-monsoon and in all other months, whereas in toads it did not alter throughout the two year period.

Conclusion

The present study infers thatserum glucose was assumes higher in frogs, whereas protein, cholesterol, urea, potassium and calcium concentration assumed higher in toads. However, sodium concentration did not alter throughout the study between the two species. It also indicates clear seasonal changes in serum composition where protein, cholesterol, potassium and calcium were higher during monsoon, whereas glucose was lower. However, urea was rose to maximum during post monsoon period in both species.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest associated with this article.

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Table 1. Seasonal changes in serum constituents of *Hoplobatrachus tigerinus* and *Duttaphrynus melanostictus*.

Serum constituent	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Monthly Changes F' Value	Interaction, Season vs Species F'' value
Glucose (mg/dl)	32.0±2.8 *Ht 20.4±0.7 Dm	40.7±4.3 22.7±1.5	37.1±2.7 23.9±2.7	36.2±2.2 27.7±1.4	6.2±2.0 27.8±2.2	18.4±0.6 10.7±0.7	14.2±1.4 12.6±0.9	15.0±0.3 21.2±2.1	16.4±2.0 22.1±2.4	29.7±3.3 26.2±1.1	34.8±3.2 22.9±1.4	31.1±3.7 20.7±1.0	16.1 10.8	6.5
Protein (g/dl)	3.5±0.1 3.0±0.1	3.1±0.2 2.5±0.1	3.0±0.1 2.4±0.1	2.6±0.1 3.2±0.4	2.7±0.1 2.6±0.2	4.2±0.2 5.9±0.2	3.7±0.2 5.1±0.2	3.6±0.4 5.9±0.2	4.7±0.2 5.9±0.3	4.1±0.1 3.9±0.2	3.0±0.1 3.1±0.2	3.4±0.1 2.8±0.1	11.0 57.1	14.0
Cholesterol (mg/dl)	43.3±9.5 51.2±3.6	50.5±5.1 54.3±6.0	40.4±3.6 43.3±5.0	55.7±0.4 53.8±1.5	3.2±2.4 56.1±6.5	0.5±16.4 93.4±14.8	0.7±20.3 83.1±8.1	64.2±2.2 110.7±21.3	3.2±15.1 6.3±12.6	35.7±2.1 40.9±2.9	43.4±4.0 52.5±6.2	46.0±6.3 47.3±3.6	1.6 4.3	1.1
Urea (mg/dl)	41.8±4.8 93.3±4.7	27.4±4.3 35.0±2.6	21.9±2.6 28.5±1.4	31.8±2.7 32.6±1.1	38.7±3.6 49.1±3.9	18.2±4.7 54.3±5.2	13.8±0.7 35.1±3.6	29.0±3.2 26.2±2.6	25.7±2.4 38.5±4.0	38.9±1.3 62.6±3.7	36.9±4.1 81.8±4.6	51.9±3.5 92.3±2.4	10.5 28.8	10.3
Sodium (mM/l)	160.7± 22.4 139.3±13.2	5.8±30.7 129.2±16.4	150.0±35.7 133.3±14.0	143.8±26.7 118.8±9.2	152.8±23.2 125.0±10.3	107.1±22.4 107.1±9.0	114.3±31.8 129.2±12.0	106.3±18.2 100.0±7.7	117.9±11.9 121.4±10.1	162.5±20.5 117.9±16.1	155.6±34.7 145.8±17.8	150.0±30.2 110.7±28.3	1.1 1.5	0.9
Potassium (mM/l)	4.5±0.2 5.9±0.5	2.6±0.2 4.5±0.4	2.7±0.2 4.9±0.6	2.6±0.2 5.2±0.5	2.0±0.0 5.7±0.3	5.8±0.2 6.0±0.3	5.5±0.2 6.2±0.2	5.4±0.5 5.9±0.3	5.0±0.2 6.1±0.2	1.8±0.2 2.8±0.2	3.6±0.3 5.7±0.4	3.6±.3 3.8±0.2	32.0 9.8	6.0
Calcium (mM/l)	6.3±0.2 7.3±0.4	8.5±0.2 6.8±0.5	7.7±0.3 9.6±0.6	7.1±0.2 7.1±0.4	6.8±0.2 8.6±0.4	7.1±0.3 7.6±0.8	7.6±1.5 8.0±0.7	7.5±0.4 9.3±0.8	8.9±0.6 9.7±0.6	6.2±0.1 7.8±0.4	6.2±0.2 8.1±0.2	6.4±0.2 7.3±0.4	6.6 3.6	3.1

*Values are mean ± SEM of 6-10 animals. Ht= *Hoplobatrachus tigerinus;* Dm= *Duttaphrynus melanostictus.*

A; Significant (P 0.01/0/001) by one way ANOVA.

b; F value of one-way ANOVA for monthly changes in each species , significant (P 0.01/0.001).

c; Interaction factor significant (P 0.01/0/001) by two way ANOVA.

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