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SEASONAL VARIATION IN NUCLEIC ACIDS, PROTEIN AND CERTAIN ENZYMES IN TESTIS AND EPIDIDYMIS OF COMMON INDIAN ROCK LIZARD, *PSAMMOPHILUS BLANFORDANUS*

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ABSTRACT
Hydrolytic enzymes play a significant role in the seasonal changes of reproductive organs of animal. In this aspect, an attempt has been made to estimate the activities of acid and alkaline phosphatase and β-D-galactosidase enzymes and macromolecular contents (DNA, RNA and protein) of testis and epididymis of male common Indian rock lizard, *P. blanfordanus*, a tropical species, in different phases of their reproductive cycle. The enzyme activities and macromolecular contents are high during breeding perirod (April to July) and low during quiescence or post-breeding period (November to December/January). The high activity of enzymes and increased macromolecular contents suggest enhanced cell proliferation and metabolic activities during breeding period necessary for spermatogenesis, sperm maturation and egg fertilisation.

INTRODUCTION

The modes of reproduction in lizards have been used to study their evolutionary tactics (Tinkle *et al.*, 1970). Reproduction in most of the vertebrates shows the phenomenon which is said to be cyclic or seasonal. Reproductive activity is species specific in case of seasonal breeders and is confirmed to a short period of the year called as "breeding season" and remains sexually quiescent during the remaining period (Lofts, 1968). The circadian rhythms serve to synchronise multiple molecular and biochemical processes with each other (Singh and Singh, 2012). Therefore, it is obvious that between breeding and non-breeding periods there is a significant change in biochemical as well as physiological activities of the reproductive tissues, *i.e.*, synthesis, degradation and regeneration *etc*. For all these changes, the enzymes in general and the lysosomal hydrolytic enzymes in particular play an important role (Moulton, 1974).

Phosphatases are enzymes that catalyse the hydrolysis of phosphoric acid ester with the liberation of phosphate ions. Depending on the pH optima at which these enzymes act, two major groups are recognised and these have been designated as acid (AcP) and alkaline (AlkP) phosphatases. Acid phosphatase is an androgen dependent marker enzyme for glandular epithelium (Tenniswood *et al.*, 1976). Alkaline phosphatase is associated with the transfer of solutes across the membrane of the cell having secretory function (Goldfischer *et al.*, 1964; Goode *et al.*, 1965; McComb *et al.*, 1979; Srivastava *et al.*, 1995) which helps in transport of glucose (Danielli, 1954) and lipid (DeSchryver-Kecskemeti *et al.*, 1991) across the plasma membrane. It is also associated with metabolism of phospholipids, phosphoprotein, nucleotides and carbohydrate, and with synthesis of protein (Srivastava *et al.*, 1995). β-D-galactosidase is important as it is involved in the catabolism of glycolproteins, glycolipids and proteoglycans containing terminal galactose. Glycosidases present in epididymis modify the carbohydrate moieties on the sperm surface and play a significant role during sperm maturation (Jones, 1978).

Nucleic acids along with other macromolecules could be considered as the most important compounds in the cell, since they are responsible for information, storage and usage (Elser *et al.*, 1996). In the reproductive organs the cell proliferation takes place along with the synthesis of DNA, RNA and proteins. In the epididymis of adult animal the cell proliferation and DNA synthesis is extremely low (Majumder and Turkington, 1976). The proteins are androgen dependent in the reproductive organs and they require androgens to maintain their normal level of expression (Chapman and Killian, 1984; Ezer and Robaire, 2002). Further, the protein concentration in lizards varies among species and the seasonal variation is significant (Chapman and Killian, 1984).

Though the reproduction in reptiles and lizards in particular is as diverse as the animals of this group itself, and first to acquire the accessory reproductive organ, like epididymis (Thompson *et al.*, 2010), the report is meager on them for the

above parameters. It has been reported in two lizards that in *Phymaturus palluma,* the acid hydrolytic activity in epididymis is intense in the non-breeding period, whereas in *Liolemus elongates* the activity of this enzyme is intense in reproductive period (Grimalt *et al.,* 1995b). Besides, there is no report on AcP, AlkP and β-D-galactosidase enzyme activities, and DNA, RNA and protein contents in the testis and epididymis of male tropical common Indian rock lizard, *Psammophilus blanfordanus* (Reptilia: Diapsida : Agamidae), which is a seasonal breeder. In the present work, these enzyme activities and macromolecular contents were estimated in the testis and epididymis of this animal throughout the year.

MATERIALS AND METHODS

Animals: Live specimens were collected from Baripada and adjoining areas (21º 6′ and 22º34′ N and 85º40′ and 87º11′ E), Mayurbhanj, Odisha. The lizards were acclimatised to the laboratory conditions at least for 15 days and the sexual maturity of the animal was ascertained by the method of Pradhan (2000).

Biochemical analysis: The animals were killed by decapitation. The testes and epididymes were dissected out and freed from fats and connective tissues and weighed in monopan balance. The tissue was homogenised (1:9 w/v) in ice cold 0.25 M sucrose solution. Proteins were precipitated with 0.1% Triton x100 and centrifuged at 800 x g. The protein contents were estimated as per Lowry *et al.,* 1951. The extraction and estimation of DNA and RNA were done as per Schneider, 1957. The macromolecular contents of the testis and epididymis were expressed as μg/g wet weight of the organ.

The activities of the enzymes were assayed directly from the crude extract obtained after homogenisation. βD-galactosidase activity was measured at pH 4.5 taking 4-nitrophenylgalacto pyranoside as per Grimalt *et al*., 1995a. Acid (pH 4.8) and alkaline (pH 10.1) phosphatase activities were estimated as per Bramley (1974) taking p-nitrophenol as substrate. The enzyme activity was expressed as μmol of substrate liberated/ minute/mg protein of the organ.

EDTA was added to the extraction medium as chelating agent. The above study was undertaken for each month of the year.

RESULTS

The testicular acid phosphatase activity was lowest during December (61.95 ± 0.93) (Table 1) which is non-breeding period of the animal. Its activity gradually increased through pre-breeding period (January/February and March) and a marked increase in July (breeding period) (135.60 ± 0.95) (Table 1) was observed. Then decreased from September to December (post-breeding period) (Table 1). Similar pattern was observed for activity of epididymal AcP. The lowest was

Table 1: Monthly (or seasonal) variation in the activities of acid phosphatase (AcP), alkaline phosphase (AlkP) and β-D-galactosidase enzymes in testis of male *Psammophilus blanfordanus*. Data are mean ± standard deviation.

Month	Number of animals (n)	AcP	AlkP	β-D-galactosidase
January	6	69.57±0.95	60.94±1.43	72.77±3.88
February	9	75.13±0.89	57.13±1.93	64.94±4.49
March	5	80.32±0.81	66.75±0.70	106.69±5.91
April	5	90.92±2.77	67.19±1.30	143.00±5.27
May	7	92.32±1.62	76.01±0.99	150.01±6.09
June	7	111.04±0.94	83.73±0.77	289.09±8.37
July	7	135.60±0.95	121.77±0.91	465.14±5.11
August	8	126.77±1.64	76.43±1.14	166.24±3.93
September	9	112.59±4.59	53.93±1.77	132.03±3.55
October	9	101.41±1.18	45.15±0.54	123.35±4.21
November	8	83.08±1.26	44.48±2.50	101.23±2.23
December	7	61.95±0.93	34.82±1.33	58.86±4.66

Table 2: Monthly (or seasonal) variation in the activities of acid phosphatase (AcP), alkaline phosphase (AlkP) and β-D-galactosidase enzymes in the epididymis of male *Psammophilus blanfordanus*. Data are mean ± standard deviation.

Month	Number of animals (n)	AcP	AlkP	β-D-galactosidase
January	6	111.26±0.78	77.38±0.73	174.01±4.11
February	9	100.37±1.23	64.44±2.58	206.62±2.20
March	5	211.44±0.95	131.48±1.35	294.50±3.77
April	5	311.02±1.48	144.05±2.59	292.83±5.86
May	7	515.36±0.98	211.26±1.01	301.40±5.02
June	7	755.33±3.26	405.80±0.86	531.58±3.52
July	7	944.68±0.95	702.84±0.88	777.67±3.64
August	8	865.46±0.79	285.82±1.17	601.82±4.03
September	9	323.83±4.78	188.30±0.60	298.94±6.42
October	9	112.01±1.97	184.59±1.39	173.81±5.15
November	8	75.47±1.85	69.21±0.59	167.34±2.79
December	7	44.52±2.29	29.35±0.60	96.73±4.03

Table 3: Monthly (or seasonal) variation in the protein, DNA and RNA contents in the testis of male *Psammophilus blanfordanus*. Data are mean ± standard deviation.

Month	Number of animals (n)	Protein (µg/g of testis wet weight)	DNA	RNA
January	6	212.99 ± 2.38	0.19 ± 0.008	0.61 ± 0.093
February	9	201.14 ± 2.17	0.13 ± 0.007	1.46 ± 0.071
March	5	307.88 ± 9.92	0.35 ± 0.023	1.41 ± 0.065
April	5	325.69 ± 15.70	0.40 ± 0.006	1.67 ± 0.049
May	7	389.71 ± 6.12	0.42 ± 0.006	1.88 ± 0.044
June	7	370.17 ± 3.64	0.47 ± 0.010	2.13 ± 0.243
July	7	429.84 ± 2.86	0.65 ± 0.008	2.96 ± 0.168
August	8	285.68 ± 3.67	0.61 ± 0.011	1.56 ± 0.046
September	9	180.67 ± 8.70	0.21 ± 0.008	0.49 ± 0.037
October	9	143.77 ± 2.93	0.16 ± 0.003	0.47 ± 0.018
November	8	162.21 ± 5.15	0.12 ± 0.003	0.44 ± 0.031
December	7	133.25 ± 2.65	0.09 ± 0.007	0.34 ± 0.004

Table 4 : Monthly (or seasonal) variation in the protein, DNA and RNA contents in the epididymis of male *Psammophilus blanfordanus*. Data are mean ± standard deviation.

Month	Number of animals (n)	Protein (µg/g of epididymis wet weight)	DNA	RNA
January	6	21.50 ± 2.24	0.09 ± 0.004	0.88 ± 0.05
February	9	19.35 ± 1.85	0.09 ± 0.003	0.86 ± 0.04
March	5	24.12 ± 1.56	0.11 ± 0.005	0.98 ± 0.03
April	5	34.38 ± 1.67	0.21 ± 0.010	1.09 ± 0.08
May	7	36.88 ± 2.61	0.52 ± 0.006	1.06 ± 0.09
June	7	40.46 ± 1.44	0.71 ± 0.007	1.23 ± 0.04
July	7	53.67 ± 1.62	0.90 ± 0.006	1.46 ± 0.04
August	8	48.56 ± 1.03	0.61 ± 0.007	1.13 ± 0.03
September	9	46.87 ± 2.59	0.34 ± 0.014	0.65 ± 0.04
October	9	33.98 ± 2.15	0.20 ± 0.005	0.51 ± 0.03
November	8	18.19 ± 1.01	0.06 ± 0.005	0.46 ± 0.04
December	7	9.83 ± 1.90	0.03 ± 0.003	0.36 ± 0.04

in December (44.52 ± 2.29) and highest during July (944.68 ± 0.95) (Table 2).

The activity of alkaline phosphatase (AlkP) and β-D-galactosidase showed more or less similar pattern to that of the activities of AcP for testis and epididymis (Tables 1 and 2). However, the expression of AcP was more than that of the AlkP (Tables 1 and 2). The lowest activities of AlkP and β-D-galactosidase in testis were 34.82 ± 1.33 and 58.86 ± 4.66, respectively in December and highest in July (121.77 ± 0.91 for AlkP and 465.14 ± 5.11 for βD-galactosidese) (Table 1). The lowest epididymal AlkP in December was 29.35 ± 0.60 and highest in July was 702.84 ± 0.88 (Table 2). The values of βD-galactosidase for the month of December was 96.73 ± 4.03 (lowest) and for July was 777.67 ± 3.64 (highest) in epididymis (Table 2).

The peak in protein, DNA and RNA contents of testis and epididymis was observed in July and lowest in December (Tables 3 and 4). These macromolecular contents gradually increased from January to reach peak values in July and then reverse trend was observed from September having lowest value in December (Tables 3 and 4). The protein, DNA and RNA contents of testis in July were 429.84 ± 2.86 µg, 0.65 ± 0.008 µg and 2.96 ± 0.16 µg, respectively and in December it were 133.25 ± 2.65 µg, 0.09 ± 0.007 µg and 0.34 ± 0.004 µg, respectively (Table 3). The respective values for epididymis were 53.67 ± 1.62 µg, 0.90 ± 0.006 µµg and 1.46 ± 0.04 µg in July and 9.83 ± 1.90 µg, 0.03 ± 0.003 µg and 0.36 ± 0.04 µg in December for protein, DNA and RNA contents, respectively (Table 4). An increase in the RNA/DNA ratio was observed from April to July and more so in June and July in the case of testis (Table 3). But in June and July it was low in the case of epididymis and more in December to March (Table 4).

DISCUSSION

Reptiles present a remarkable sexual cycle consisting of a period of reproduction followed by a non-reproductive period and the germinal epithelium undergoes massive involution in the period of sexual inactivity (Macola *et al.*, 1984). In the male *P. blanfordanus*, the histological study on reproductive organs shows same pattern (personal observation) as reported for other lizards (Sanyal and Prasad, 1967; Shanbhag *et al.*, 2000). Grimalt *et al*. (1995b) have reported the annual variations of acid hydrolases in the epididymis of *Liolemus elongates* and *Phymaturus palluma*. They have shown that in *L. elongates* the activity was intense in the reproductive period and in *P. palluma* the acid phosphatase was high during the post-nuptial period. Analogous fashion of acid phosphatase action is seen in testis of *Psammophilus dorsalis* and its intense activity is seen in epididymis before reproduction (Bhagya, 1993). Such phenomenon is correlated with the reproductive

cycle of *Calotes versicolor* (Ananthalakshmi, 1992). The present findings showed the highest activity of acid phosphatasse in both the testis and epididymis during breeding period (April to June/July) and there is gradual decrease in its activity till October before reaching lowest in December (Tables 1 and 2). The results suggest their possible involvement in spermatogenesis and sperm maturation as reported for other animals (McComb *et al*.,1979; Stinchi *et al*., 1998;Tulsiani *et al*., 1998).The gradual decrease in its activity instead of abrupt change suggests its role in removal of spermatozoa remaining after reproductive phase as has been suggested for certain fishes where acid phosphatase activity is high in quiescence period (Porawski *et al*., 2004).

The main function of alkaline phosphatase in testis is to transport various metabolites from Sertoli cells to different germ cells and helps in differentiation and proliferation of germinal epithelium (Tice and Barnett, 1963). The increased activity of alkaline phosphatase during reproductive phase in testis and epididymis of *P. blanfordanus* (Tables 1 and 2) during reproductive phase is attributed to the above functions. The decrease in alkaline phosphatase activity may result in altered transport and an inhibitory effect on the cell growth and proliferation (Goldfischer *et al*., 1964). The decreased activities of alkaline and acid phosphatase indicate disturbance in the structure and integrity of cell organelles, like endoplasmic reticulum and membrane transport system (Nchumbeni *et al*., 2007). As the activity of alkaline and acid phosphatase decreases in testis and epididymis during non-breeding period (Tables 1 and 2) it halts their growth and results in quiescence.

The findings on the above two enzymes corroborate with the finding of Wislocki (1949) on the testis and epididymis of Virginia deer (*Odocoileus virginianus borealis)* and Japanese deer (*Cervus nippon*). The acid and alkaline phosphatase activities in these two deer are more in the fall (October and November; rutting season) with active spermatogenesis. The enzymes are very less in both the tissues or almost absent in epididymis during spring (June; non-breeding period) when spermatogenesis is in abeyance.

The glycosidases in the testis and epididymis cause modification of the membrane (Tulsiani *et al*., 1995). Although the actual mechanism are not yet clear, it is possibly that the membrane associated glycosidases that play a significant role throughout the life history of spermatozoa to modify sperm surface and provide potential signals which enables the transfer of biological information (Olden *et al*., 1985). As per previous studies increased activity of β-D-galactosidase in the testis and epididymis results in modification of sperm membrane glycoproteins and releases free galactose. It has been mentioned that free galactose act as a source of carbohydrate for energy requirement of sperm and trigger membrane related events leading to the acquisition of motility and fertility by sperm (Hall and Killian, 1987; Talbot *et al*., 2003).

The DNA contents vary in various stages of spermatogenesis. The amount of DNA in the primary spermatocyte, secondary spermatocyte and spermatid are generally present in the ratio of 4:2:1 (Davson and Segal, 1975). However, in the present study whole of testis and epididymis were used for quantification of DNA contents. It was observed that DNA contents for both the organs are highest during breeding period (April to July) (Tables 3 and 4). Similar pattern was observed for both protein and RNA contents of testis and epididymis (Tables 3 and 4). Increase in all these macromolecular contents during reproductive phase suggests more proliferative and cellular metabolic activities of these organs during this phase. The increase in RNA to DNA ratio in the testis during breeding period support the view that there is increase in cellular metabolic activities. The less DNA contents during non-breeding period may be attributed to the atrophy of the organs (changes in the number of cells) during quiescence (post-breeding) and early stage of recrudescence (pre-breeding). In another lizard, *Lacerta vivipara,* it has been reported that during regression phase (post-mating atrophy) there is decrease in quantity of protein and loss of organ weight (Ravet *et al*., 1987). The increased protein content of reproductive tissues of this animal during breeding season is due to influence of alkaline phosphatase during that period as this enzyme is involved in the synthesis of protein (Srivastava *et al*., 1995). Sreekala and Zutshi (2010) are of the opinion that decrease or increase in the liver acid and alkaline phosphatase activity of *Labeo rohita* from polluted and non-polluted freshwater lakes is very much in correlation with protein content of liver.

From the above discussion it is concluded that the fluctuations in enzymatic and macromolecular profiles of testis and epididymis plays an important role in cell proliferation, spermatogenesis, sperm maturation , egg fertilisation, transport system, and metabolic activities (synthesis and degradation of molecules) during different phases of its reproduction, *i.e.*, highest during breeding period (April to June/July or sometimes to early August), lowest in post-breeding (November to December/ January) and intermediate value during pre-breeding period (January/ February to March).

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