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MOLECULAR AND TISSUE MODELLING ROLES OF ALKALINE PHOSPHATASE IN REPRODUCTIVE ORGANS OF BAT *Cynopterus sphinx (vahl).*

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ABSTRACT:
The enzyme alkaline phosphatase is a hydrolytic enzyme that is enzyme elaborated by a combined efforts of the GERL complex in cells of reproductive organs of animals and humans under the influence of sex-hormones (testosterone, estrogen). This enzyme shows periodical fluctuations according to the various stages of the reproductive cycle, especially the copulatory cycle when it is secreted on higher quantity coinciding with the first active breeding period (Oct-Nov) when its value is nearly twice to that recorded at the second active breeding period (Feb-March) in the bat, Cynopterus sphinx (vahl) a fruit consuming bat that is widely distributed in Indian subcontinent. During prebreeding season (Aug- Sep) the synthesis and secretion starts elevating, and in post-breeding season it starts declining to reach a level that is low and matches with the absence of spermatogenesis in the testes. The accessory sex organs follow the above trend in production of this enzyme during other phases the reproductive cycle. Alkaline phosphatase is absent in nonsecretory tissues, their presence in sex-organs have been linked to transport of sugar across the cells, thereby helping in secretory processes, maintenance of functional inter-relationship between these organs. The enzyme is androgen dependent in the testes and accessory sex organs. One can hypothesize presence of genes in the gonadotrophs present in pituitary gland as well as in testes interstitial and germinal cells, if not in sex- organs. A feedback mechanism may be regulating formation of more lysosome populations of hydrolytic organelles during the entire reproductive cycle of this bat for modelling of cells as well as digesting the residual unwanted structures to be reused again to commence a new mitotic – meiotic turn – over cycles again to help successful continuation of and existence of the species.

Keywords:- Testis, accessory sex organs, alkaline phosphatase, Cynopterus, Hormone ,Biochemistry.

INTRODUCTION :

There is severe paucity of information regarding alkaline phosphatase enzyme biochemistry associated with the testes and accessory reproductive organs of the aerial mammals, the bat Cynopterus sphinx is one of them. So, the this investigation was undertaken to determine differences in the levels of enzyme. Although such work have been done in other mammals under normal and experimental conditions.

MATERIAL & METHODS:

The male specimens of the Indian fruit bat Cynopterus sphinx (vahl) were collected throughout the year to study histoarchitecture, histoenzymological changes and biochemical alterations in the testis and the accessory sex organs during the reproductive cycle of this bat.

The bats were collected with the help of butterfly nets from the dried fronds of the palm trees in and around Kolhapur (Western Maharashtra) during January 1996 to March 1998. Live animals were brought to the laboratory. The animals were anaesthetized by ether and their weight measured on spring balance. The male reproductive system was removed, cleaned of fats and connective tissue. Testis, epididymis, seminal vesicles, prostate and Cowper's glands were separated and were

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weighed to the nearest 0.1 mg on a Mettler Balance.

ESTIMATION OF ALKALINE PHOSPHATASE:

Alkaline phosphatase was estimated by the method of King and King (1954). This method utilises the reaction of 4 aminoantipyrene with phenol even in presence of proteins thus eliminating the step for separation of proteins.

A) Reagents: Carbonate-bicarbonate buffer (0.1M, pH 103 at 37°C) (Delong and King, 1945)

I) 10.6 gm chemically pure anhydrous sodium carbonate was dissolved in distilled water and the volume was made to one litre. 2.1 gm of chemically pure sodium bicarbonate was dissolved in 250 ml of distilled water. These two solutions were mixed to get required buffer.

II) Substrate disodium phenyl phosphate (0.02M): 0.436 gm of anhydrous sodium phenyl phosphate properly dried in a dessicator was dissolved in 100 ml of distilled water. As this solution does not keep well even in refrigerator it was prepared fresh for every experiment.

III) Sodium hydroxide (0.5N): 2.0 gm of sodium hydroxide dissolved in 100 ml of distilled water.

IV) Sodium bicarbonate (0.5N): 4.2 gm of pure dry salt dissolved in 100 ml of distilled water.

V) 4-aminoantipyrene :0.6 gm of 4-aminoantipyrene dissolved in 100 ml of distilled water.

VI) Potassium ferricyanide : 2.4 gm o f potassium ferricyanide dissolved in 100 ml of distilled water.

VII) Standard phenol solution: Stock solution: 100 mg of freshly crystalline phenol dissolved in 200 ml of 0.1 N HCI.

Working standard: 1 ml of stock standard solution was diluted to 100 ml with distilled water just before use.

B) Extraction of alkaline phosphatase: Homogenates were prepared in cold 0.9% saline, centrifuged for 10 minutes at 2000 RPM and supernatant was used as enzyme extract. **Procedure**: To 1 ml of buffer 1 mi of substrate was added and preheated for 5 minutes at 37°C. Two tubes of every homogenate (test and control) were taken. Enzyme extract was added in the tube for the test reading only and all tubes were incubated for 15 minutes at 37°C. For standard and blank 1 ml each of working standard and distilled water were added respectively. After incubation 0.8 ml of 0.5 N sodium hydroxide and 1.2 ml of sodium bicarbonate were added to all tubes. After this 0.1 ml of enzyme extract was added to control readings, 1 ml each of 4-aminoantipyrene and potassium fermicyanide were added to all tubes, contents were mixed well and readings were taken at 520 nm.

U (for unknown) for test against control and S (standard) for standard against blank.

Activity of enzyme was calculated in terms of King Armstrong units (KA units) per 100 ml of enzyme extract according to the following formula.

King Armstrong units per 100 ml = x 0.01 x 100 x S 01x10 IU. Per litre(KA units) per 100 ml x 7%. Statistical analysis of biochemical results.

S.E.M = Standard deviations estimated from the mean.

$$SEM = \frac{\sqrt{\sum(X1 - \bar{X})^2 + (X2 - \bar{X}) + (X3 - \bar{X})}}{N - 1}$$

X1, X2 x X3 = individual value X = mean N no of observation (N = 3)

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RESULTS AND DISCUSSION:

Differentiation of germinal epithelium and germ cells, steroid synthesis according to requirements, shuttling of molecules between Sertoli cells and germinal cells, inorganic phosphate liberation from ester organic compounds may be the energy source that favour formation of mature spermatozoa are some of the functions associated with alkaline phosphatase in the bat, Cynopterus. According to (D. R.Saxena and A.K. Kawadkar) the investigators other functions of this enzyme is lysis of unwanted tissue debris in the genital system, recycling and reutilization of various molecules in the next mitotic- meiotic cycles. This seems vital for maintaining the material - energy budget for anabolic and catabolic functions. Certain genes hypothesized as "GASOG" (gonad accessory sex organs gene) may be controlling sequential, properly timed reproductive cycle in time and space in human's and animals. The packing of sperm bundles and viability after acquiring fertilizing ability depends on the duration of sperms storage in the cauda epididymis and its release during copulation when optimum hormones are circulating in the reproductive system.

Human semen contain very less, but on the contrary semen of bull, boar and rabbit exhibit high level of alkaline phosphatase (Haq and Mullen 1948,; Reid, Ward and Salisbury 1948 a; Bell and Lake 1962 b).In the bull high alkaline phosphatase appear to have relationships with metabolism as well as high motility and viability of the spermatozoas (Rousell and Stallcup 1966).

More alkaline phosphatase in comparison to acid phosphatase was biochemically estimated in the testes of male albino rats (Rao et al. 1979), Coypu (*) and least in the palm squirrel, Funambulus pennanti (Saxena and Gadegone 1996).

The testicular enzyme is expressed as King Armstrong units, this metabolite level is low 24.56 – 25.62 in May – July when the bats, Cynopterus were in inactive or in a quiescent condition.The enzyme secretion increased slowly to 36.48 – 51. 25 in August – September coinciding with recrudescence period when spermatogenesis resumes. The testicular alkaline phosphatase value being 76.59 – 89.42 KA units that parallel the high level of male hormone testosterone during the first peak (October – November), it later declines to 40.90 – 33.16 during the intervening period in December – January. Later the enzyme increased to 61.70 – 70.20 during second active breeding period in February – March but is less than the first peak period. When testicular regression follows in April the amount of alkaline phosphatase recorded is 30.6 KA units. Cyproterone acetate (an antiandrogen) treat,ment for 60 days reduced alkaline phosphatase secretion in adult male Wistar rats, but more decrease occurred in acid phosphatase secretion (Menon et al.,1988.

More alkaline phosphatase is secreted in corpus and cauda epididymis of monkey than in caput part (Riar et al, 1972). Cauda epididymis contributed greater alkaline phosphatase than the enzyme acid Phosphatase in rabbit as reported by Jones Glover, 1973). Alkaline phosphatase is produced in caput epididymis of loris (Manjula, 1980), in the ductuli efferentes, caput, corpus and cauda epididymis of mature goat (Capra hircus), more amount of alkaline phosphatase occur in cauda epididymis of goat (Chauhan and Sharma, 1988).

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In the bat, Cynopterus sphinx maximum amount of alkaline phosphatase is synthesized for reproductive facilitation in the cauda epididymis in comparision to minimum amount elaborated by in comparison by Cowper's as well seminal vesicle. Although, fluctuations of this enzyme was recorded , high activity during the first and second activities of copulatory cycle indicates differential functional significance correlates with higher density as well as healthy morphology-functionally active spermatozoas when the male sex hormone testosterone levels were higher. (See table No 1. and the histogram No 1). According to Sapkal (1986), in the epididymis of the bat, Rousettus leshenaulti cyclical changes occurred in the levels of alkaline phosphatase, one in October and the second peak period in January, but it declined in April to become low in April – July when spermatogenesis ceased. Similarly, high enzyme activity in the caput and cauda epididymis was reported during the first and second active breeding than in the quiescent period in Funambulus pennanti in comparison to other reproductive organs (Saxena 1996 and Gadegone).

The seminal vesicles of this bat elaborate less alkaline phosphatase, it was very low (5.64-9.21) during sexually quiescent period (May–July), increased during recrudescence period and elevated during first active breeding period (30.39-42.42). After a down trend the enzyme increased during the second active breeding period.

Manjula (1980) reported high amount of Alkpase in prostate of Loris tardigradus, whereas the same gland produced low concentration of this enzyme in case of plain mouse (Psuedomys australis) as well as in the hopping mouse , Notomys salixis (Tsonis et al., 1981). A reverse trend of low enzyme secretion was reported in human by (Mann and Lutwak – Mann 1951).A significant drop in enzyme secretion was evident in the prostate of Loris (Manjula 1980).. The prostate gland ranks third in order of enzyme contribution to semen in the bat, Cynopterus sphinx ; it exhibits cyclic changes being the highest during the first and second active seasons of breeding period, but lowest in quiescent period (May – June) as well as in post – breeding period in April.The enzyme slowly elevated with onset of spermatogenesis in the pre – breeding season.

Cowpers gland.

In Cynopterus the Cowper's gland contribute the least alkaline phosphatase. The amount of enzyme varied with the activity in the testes of this bat paralleling the hormone levels. The first and second active breeding seasons exhibit higher enzyme levels that dipped low in quiescent period in May – June and also in post – breeding stage in April. In the pre – breeding stage in Aug – September the enzyme level slowly increased.

Alkaline phosphatase alone or in combination with Acpase, SDH, ATPase, esterases, as well as non enzyme substances like sialic acid, ascorbic acid, cholesterol, proteins, minerals, etc., may be performing all dynamic functions at levels like the molecular, synthesis of compounds and hormones at the cell, tissue organ and organ system and the fluids secreted therein for perpetuation of species by regulating the fertility of animals.

CONCLUSION :

In the bat, Cynopterus sphinx, alkaline phosphatase enzyme perform several functions in the testes and accessory sex-organs, its level, modifies depending on the the stages of breeding cycle under the influence of

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hormones produced by the pituitary- testes endocrine glands.

When spermatogenesis is absent alkaline phosphatase levels is lowest, it is highest during both active spermatogenesis periods. The cauda epididymis, caput epididymis, prostate, testis, seminal vesicles and Cowper's gland contribute in decreasing order alkaline phosphatase in the biochemical composition of the semen to maintain the integral structure function and the chemical environments of the reproductive organs.

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