CHAPTER - VI

SEASONAL ALTERATIONS IN THE ACTIVITY OF PHOSPHOMONOESTERASES AND PROTEIN CONTENT IN THE GONADS OF NORMAL AND PINEALECTOMISED DOMESTIC PIGEONS, *COLUMBA LIVIA*

Nonspecific phosphomonoesterases (acid and alkaline phosphatases) are found widely distributed in almost all animal tissues. Apart from their ubiquitous distribution, quite a bit of functional versatality is also exhibited by them. Though primarily involved with hydrolysis of phosphate esters and dephosphorylating mecanisms, phosphomonoesterases are also implicated in many physiological processes like hydrolysis, cellular phagocytosis, protein synthesis, regulation of pyrixoxyl phosphate requiring enzymes, Vit. B6 metabolism, absorption and phosphorylation, steriod transport, lipid metabolism (acid phosphatase : Ghiretti, 1950; Weber and Nichus, 1961; Friede, 1964; Steetan, 1964; Strauss, 1964; Andrews and Turner, 1966; Dipietro and Zengerle, 1967; Pearse, 1968; Klockars and Wagelins, 1969; Heinrikson, 1969; Blank and Synder, 1970; Cohen, 1970) and transmembrane transport mecanisms, calcification, formation of fibrous protein, growth, differentiation, DNA metabolism, carbohydrate metabolism (Alkaline phosphatase: Moog, 1946; Sols, 1949; Bradfield, 1951; Rosenthal *et al.*, 1960; Rogers, 1960; Simkiss, 1964; Raekallio, 1970). Inspite of the many functions cited above, relatively less information is available

regarding their specific biological functions. It is now realized that these enzymes occur in different isozymic forms which are sensitive to hormones and other factors. Functional involvement of phosphatases in gonads and accessory sex organs has also been reported (Kugler et al., 1956; Bialy and Pincus, 1967; Chinoy et al., 1973; Chinoy and Sheth, 1977; Sheth and Chinoy, 1977). In this light precise time specific activity of these enzymes could be of importance in seasonal gonadal activities, which on this premise should be under the regulative influence of the hypothalamo-hypophysio-gonadal endocrine axis or a related one. Since pineal is shown to influence this axis and affect the seasonal periodicities of gonads, it would be interesting to see the possible alterations if any in the activity levels of phosphomonoesterases in the gonads on a seasonal basis post-pinealectomy.

Though the protein content of tissues remain more or less constant under normal circumstances, organs undergoing seasonal functional alterations could be expected to show some variations in their protein content. Such alterations might affect either the structural proteins or the enzymic proteins or even both and thereby alter the total protein content of a tissue. As the gonads represent organs which depict tremendous variation in their structural components and metabolic features during their active and inactive states, changes in their total protein contents could be considered as a corollary. Again,

pinealectomy induced alterations in the protein content of the gonads would be informative in view of the observed and reported influence of pineal on gonadal functioning. Hence in the present study evaluation of total protein content and the activity levels of phosphomonoesterases have been attempted in the gonads of normal and pinealectomised domestic pigeons on a seasonal basis.

MATERIAL AND METHODS

Domestic pigeons obtained from the local animal dealer were maintained in the laboratory for acclimation and divided into 3 experimental groups (C, PN, PX) as outlined in the previous chapter. Pineal ablation was performed during the two reproductive phases and the birds were then sacrificed along with the corresponding intact and sham operated controls on 30, 45 and 60 days post-surgery. The gonads were removed, blotted dry and processed for the biochemical assay of acid and alkaline phosphatases employing the method described in sigma technical bulletin No.104 using para nitrophenyl phosphate as the substrate. The protein concentration in the tissue homogenates was estimated by the method of Lowry *et al.* (1951). Enzyme activities were expressed as μ moles paranitrophenol released/mg protein/ 30 mints. and the protein content as percentage of tissue weight.

RESULTS

The changes in the protein content and activity of phosphatases in the gonads during the breeding and post-breeding phases of normal and pinealectomised birds are shown in table-1 and figures 1-3.

CHANGES IN NORMAL BIRDS

The average total protein content of gonads was relatively higher during the post-breeding phase than during the breeding phase. The increase in the protein content post-breeding being about 17 % . On a comparative basis the level of acid phosphatase activity was higher than that of alkaline phosphatase. Both the enzymes had higher activity levels during the breeding season than during the non-breeding season. Between the two, the difference in the activity level (vis a vis the two phases) was more significant for alkaline phosphatase than that for acid phosphatase. Whereas the percentage decrease for acid phosphatase was about 6 %, that for alkaline phosphatase was about 26 % .

CHANGES DUE TO PINEALECTOMY

Pinealectomy during the two breeding periods brought about differential changes in the protein content as well as in the activity of phosphatases. Whereas the protein content was increased post-pinealectomy, the activity level of acid

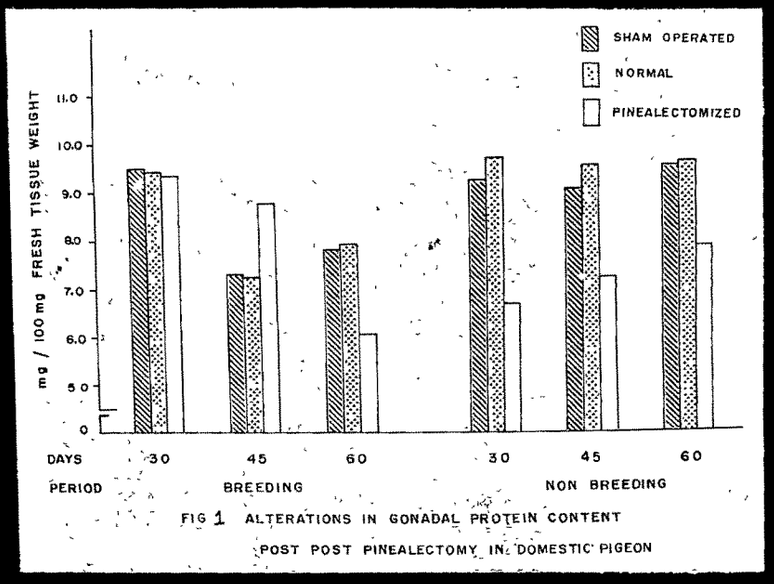

FIG 1 ALTERATIONS IN GONADAL PROTEIN CONTENT POST POST PINEALECTOMY IN DOMESTIC PIGEON

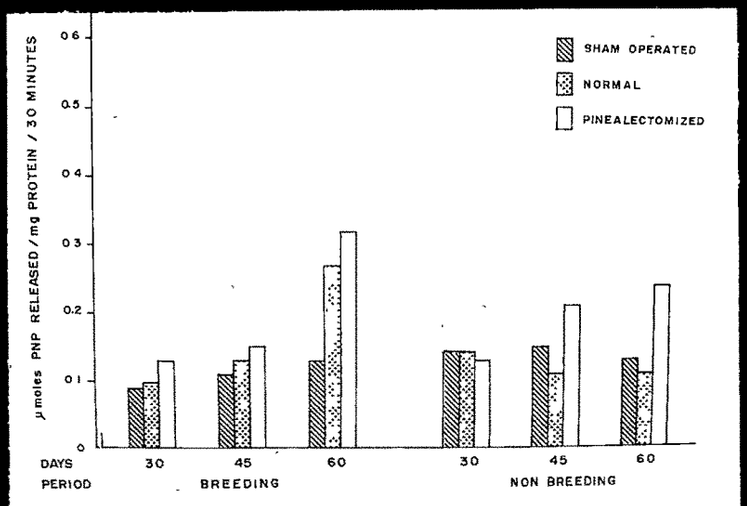

FIG. 2 ALTERATIONS IN GONADAL ALKALINE PHOSPHATASE ACTIVITY POST PINEALECTOMY IN DOMESTIC PIGEON

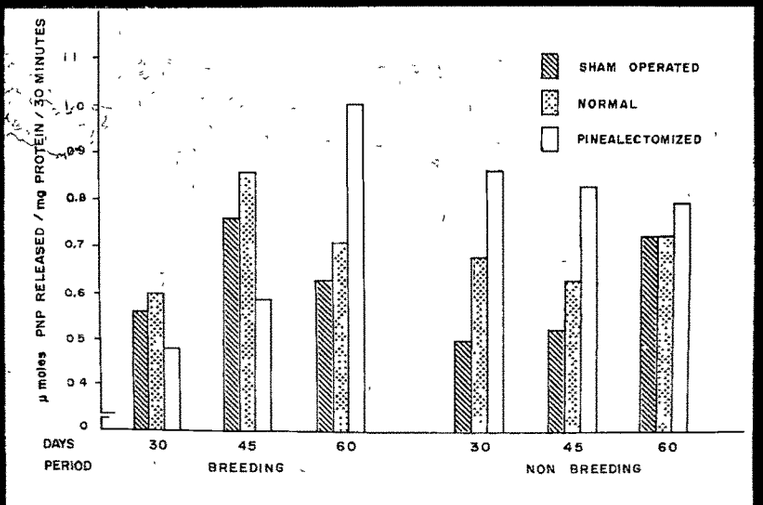

FIG. 3 ALTERATIONS IN GONADAL ACID PHOSPHATASE ACTIVITY POST PINEALECTOMY IN DOMESTIC PIGEON

phosphatase was decreased during the breeding season. The level of alkaline phosphatase was however increased slightly. In the non-breeding pinealectomy brought about a decrease in protein content and increase in activities of both the phosphatases. The overall variation in the protein content ranged between 20-30 % same as that for acid phosphatase. However the increase in alkaline phosphatase activity during the post-breeding phase was nearly 100 % .

DISCUSSION

From the results it is apparent that the gonadal protein content remains lower during their active phase. Concomitantly, the activity of both the phosphatases is increased. Hence the breeding phase whence the gonads are active in steroidogenesis and spermatogenesis is marked by increased levels of activity of phosphatases and decreased protein content. The reverse set of changes appears to be true for the non-breeding phase whence the gonads are inactive in spermatogenesis as well as steroidogenesis. A couple of points of interest worth noting with regard to the activity of phosphatases are (1) the activity of acid phosphatase is comparatively higher than that of alkaline phosphatase in general. (2) The variation between seasons on a percentage basis is more marked for alkaline phosphatase than for acid phosphatase. From these, it could be presumed that acid phosphatase is more important for the overall functional aspects of

the gonads and alkaline phosphatase is involved in more specific functional events associated with breeding. This seems to be true for wild pigeons too (Patel, 1982; Ayyar, personal communication). However, Dasgupta and Bhattacharya (1984) have shown tremendous variation in gonadal acid phosphatase activity between the breeding and non-breeding months in the red vented bulbul. Whereas the acid phosphatase activity could be associated with functions like protein synthesis, steroid transport and lipid metabolism (Dipietro and Zengerle, 1967; Blank and Snyder, 1970), alkaline phosphatase activity could be implicated in nucleic acid synthesis, phospholipid metabolism and transfer of metabolites and phosphates (Chavremont and Firket, 1953; Allen and Slater, 1956; Danielli, 1954), events which are all of immense significance in an active gonad. The reduced activity of these enzymes in the non-breeding period could in this light suggest loss of the above functions in the regressed gonad.

Seasonal alterations in gonadal activity are essentially due to variations in the secretion of gonadotrophic hormones from the pituitary by mechanisms which are far from clear affecting the hypothalamo-hypophysio-gonadal axis. In most of the seasonally breeding animals such mechanisms are also known to alter the secretion of other endocrines probably in a secondary fashion. The sum total of such changes is to orient the animal's physiology in keeping with the reproductive status. Predictably,

the altered endocrine status could be considered to be responsible for bringing about alteration in the activity levels of various enzymes in the gonad. Pineal is known to be capable of affecting the hypothalamo-hypophysio-gonadal axis as well as other endocrine glands, and has been shown to have a progonadal as well as antigonadal role in domestic pigeon (Chapter 1). In keeping with this role, pinealectomy brings about gonadal regression during the breeding season and gonadal activation during the non-breeding season. The present observations on protein content and activities of phosphatases in gonads of pinealectomised birds during the breeding season have shown changes characteristics of normal birds in the non-breeding season and vice versa in birds pinealectomised during the non-breeding season. Reduced levels of the enzymes and increased protein content in the gonads of pinealectomised birds during the breeding season, and increased activity of enzymes and reduced protein content in the gonads of birds pinealectomised during the non-breeding season are in keeping with the regression and active conditions of the gonads respectively : Changes characteristic of normal birds in the non-breeding and breeding phases respectively. From the changes observable in normal birds as well as pinealectomised birds, it is obvious that reduced protein content and increased activities of both acid and alkaline phosphatases are linked with active gonads while reverse changes are identifiable with inactive gonads. Similar observations are also made by Patel (1982) in normal

and pinealectomised wild pigeons. Presumably, alkaline phosphatase can be accredited with the function of transport of metabolites and other vital substances on a selective fashion (as there is an effective blood-testis barrier) and acid phosphatase with germ cell differentiation and sperm metabolism, events which form the crux of spermatogenically active gonads. Functional significance of acid phosphatase of a similar nature has been suggested by Dasgupta and Bhattacharya (1984) in the red vented bulbul. A natural query in the present context is whether the changes observed are due to alterations in gonadotrophic hormones themselves or are they due to the alterations in other endocrine secretions ? . This is more pertinent as changes in both thyroid and adrenal (parallel to gonadal activity) have been noted to occur in relation to gonadal activation and regression (Chapter 1). It is likely that the hypothalamo-hypophysio-gonadal axis works in conjunction with other endocrines to bring about gonadal activation and regression along with the associated biochemical and metabolic changes.