CHAPTER 8

SEASONAL ALTERATIONS IN ACTIVITIES OF NON-SPECIFIC ACID AND ALKALINE PHOSPHATASES AND PROTEIN CONTENT OF NORMAL AND PINEALECTOMIZED FERAL BLUE ROCK PIGEON, *COLUMBA LIVIA*.

Of the various phosphatases, acid and alkaline phosphatases are the most widely distributed in animal systems. These non-specific phosphatases are supposed to be involved in a variety of cellular activities depending on the site of localization. It has been reported that hormones influence activity of these non-specific phosphatases in rats and chick (Manwell and Betz, 1966; McWhinnie and Thommes, 1973; Yeh and Moog, 1977; Wilfred and Rao, 1977). Alterations in acid phosphatase activity or its distribution were studied in many pathological conditions. Acid phosphatase is associated with various important activities such hydrolysis, cellular phagocytosis, protein synthesis,

vitamin B6 metabolism, absorption, steroid transport, phosphorylation and lipid metabolism. (Strauss, 1964; Andrews and Tarner,1966; Di Pietro and Zengerle,1967; Pearse,1968; Klockars and Wegelius,1969; Heinrikson,1969; Blank and Snyder,1970). Whereas acid phosphatase is active at acidic pH, alkaline phosphatase is active at alkaline pH and is involved in calcification, transport of metabolites across cell membranes, formation of fibrous protein, growth, differentiation, DNA metabolism and carbohydrate metabolism (Moog,1946; Sols,1949; Bradfield,1951, Rosenthal et al.,1960; Rogers,1960; Simkiss, 1964; Raekallio,1970).

Localization of non-specific phosphatases in the liver and kidney of various species of birds has been studied (Shah et al., 1972a, 1976). Presence of these enzymes in sex accessory organs has also been documented. Some reports show influence of sex hormones on the activity of these phosphatases (Ambadkar and Gangaramani, 1979). Though, considerable attention has been given to the non-specific phosphatases due to their involvement in cellular metabolism, relatively less specific information is available regarding their biological functions. These enzymes also occur in several enzymic forms some of which are sensitive to hormones and other factors.

About one-fifth of the fat-free body of birds and mammals is protein. Because of variations in body fat content

which are partially balanced by adjustments in water content, whole body protein is somewhat more variable. Except a few studies on protein metabolism in domestic species of birds, no data is available on protein metabolism of wild species. The importance of structural proteins in various endocrine and enzyme system is well known in all vertebrate calsses. To study the influence of pineal on the activity of acid and alkaline phosphatases and on protein content, quantitative assessment of all the three in some tissues of normal and pinealectomized wild pigeons are undertaken during the three reproductive phases.

MATERIALS AND METHODS

Wild pigeons obtained from the local animal dealer were maintained in the laboratory and divided into three experimental groups as outlined in chapter one. Pineal ablation was done during different reproductive phases as per the procedure described previously and were then sacrifised along with the corresponding intact and sham-operated controls, on 30, 45 and 60 days post-surgery. Small pieces of pectoral muscle, liver, kidney and gonad were removed, blotted and processed for biochemical assay of acid and alkaline phosphatase activity employing the method described in Sigma Technical Bulletin No.104 using

p-Nitrophenyl phosphate as the substrate. Protein concentrations in the tissue homogenates were determined by the method of Lowry et al. (1951). Enzyme activities were expressed as u mole p-Nitrophenol released / mg protein / 30 minutes and protein content as percentage of fresh tissue weight.

RESULTS

CHANGES IN NORMAL BIRDS:

Acid phosphatase activity was high in kidney and liver followed by gonads and muscle in that order (table 1; Fig.1). Kidney showed highest alkaline phosphatase activity, while in muscle and liver it was lowest. Average protein content of liver, kidney and muscle were more or less same (table 3; Fig.3). Recrudescence and breeding periods were marked by higher levels of acid phosphatase activity in all the tissues under study while it was low during regression phase. Alkaline phosphatase also showed increased activity during recrudescence and breeding in muscle, kidney and gonads while in liver, maximal activity was expressed during breeding and regression (table 2; Fig.2). Average protein content showed a gradual decrease from recrudescence to regression through breeding in liver and kidney while in muscle it was maximal during recrudescence and minimal during breeding. Protein content of gonads was maximal during regression which showed a gradual decrease through recrudescence to breeding.

EXPLANATIONS FOR FIGURES

Figs. 1 to 3 : Histogrames showing acid and alkaline phosphatase activity and protein content in three different intervals post-pinealectomy.

Fig. 1a - Acid phosphatase in liver

Fig. 1b - Acid phosphatase in muscle

Fig. 1c - Acid phosphatase in kidney

Fig. 1d - Acid phosphatase in gonad

Fig. 2a - Alkaline phosphatase in liver

Fig. 2b - Alkaline phosphatase in muscle

Fig. 2c - Alkaline phosphatase in kidney

Fig. 2d - Alkaline phosphatase in gonad

Fig. 3a - Protein in liver

Fig. 3b - Protein in muscle

Fig. 3c - Protein in kidney

Fig. 3d - Protein in gonad.

Abbreviations:

S - Sham operated.

N - Normal unoperated.

P - Pinealectomized.

FIG 1a

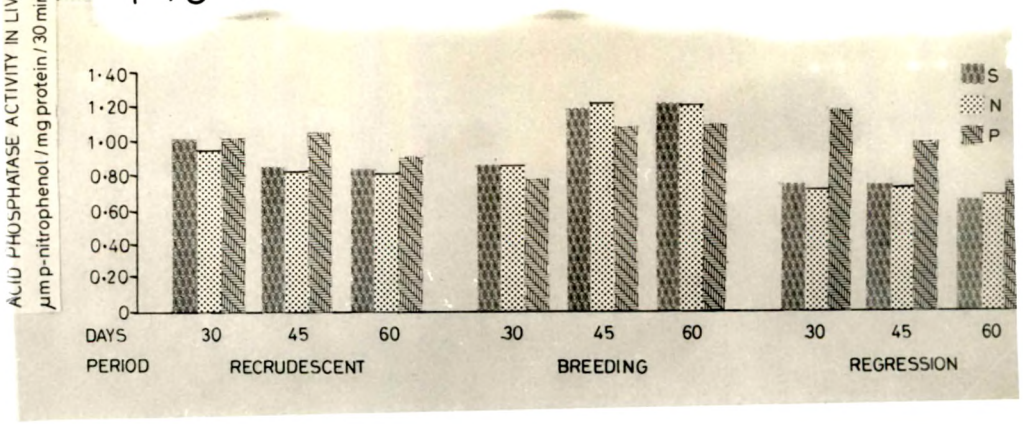

ACID PHOSPHATASE ACTIVITY IN LIVER
µm p-nitrophenol / mg protein / 30 minutes
1·40
1·20
1·00
0·80
0·60
0·40
0·20
0
S
N
P
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG 1b

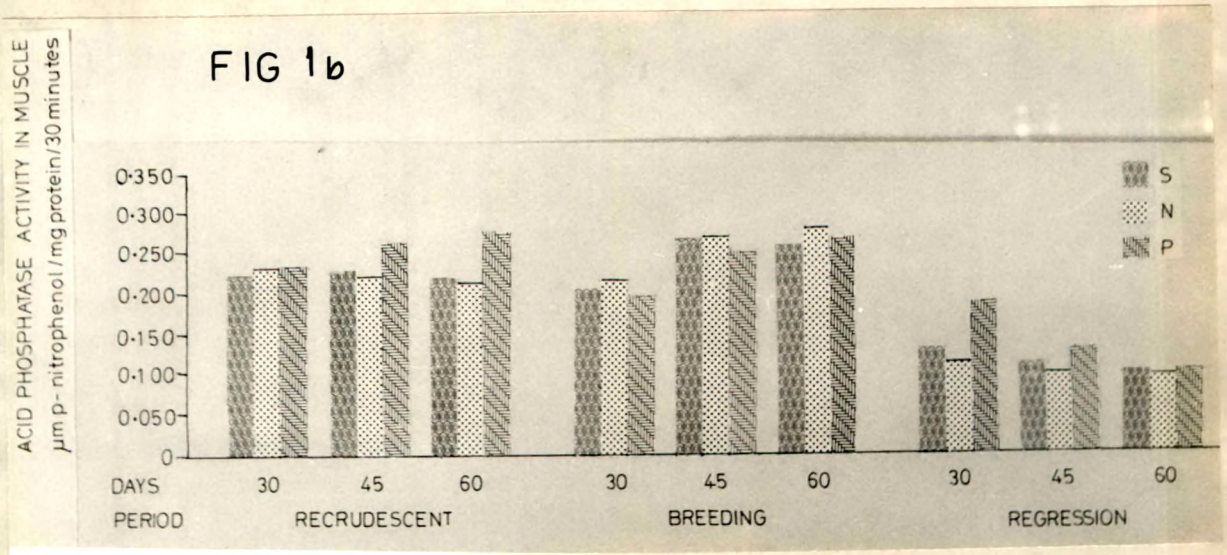

ACID PHOSPHATASE ACTIVITY IN MUSCLE
µm p-nitrophenol / mg protein / 30 minutes
0·350
0·300
0·250
0·200
0·150
0·100
0·050
0
S
N
P
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG 1c

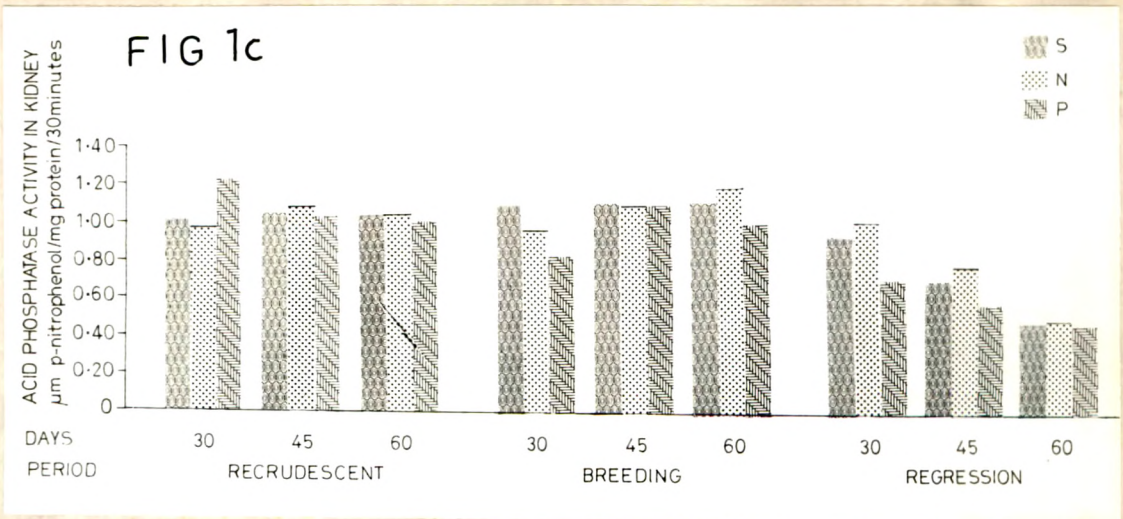

ACID PHOSPHATASE ACTIVITY IN KIDNEY
µm p-nitrophenol/mg protein/30minutes
1·40
1·20
1·00
0·80
0·60
0·40
0·20
0
S
N
P
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG 1d

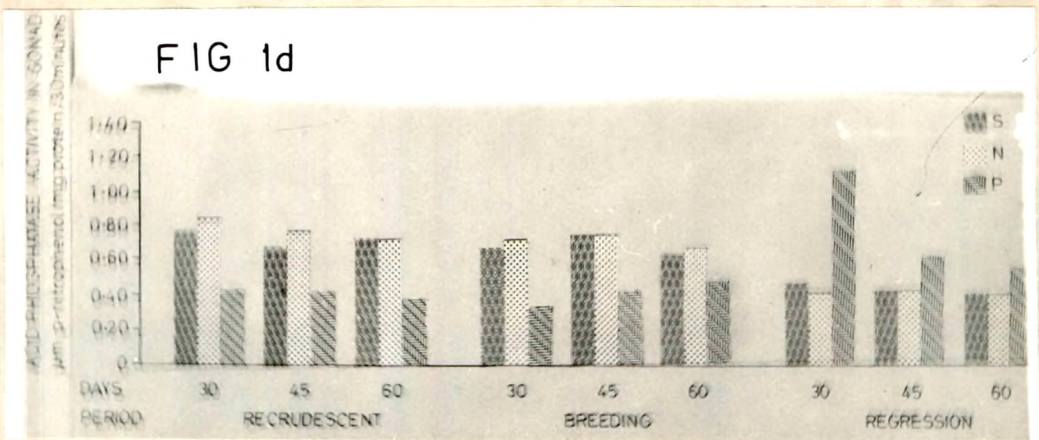

1·60
1·20
1·00
0·80
0·60
0·40
0·20
0
S
N
P
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG 2a

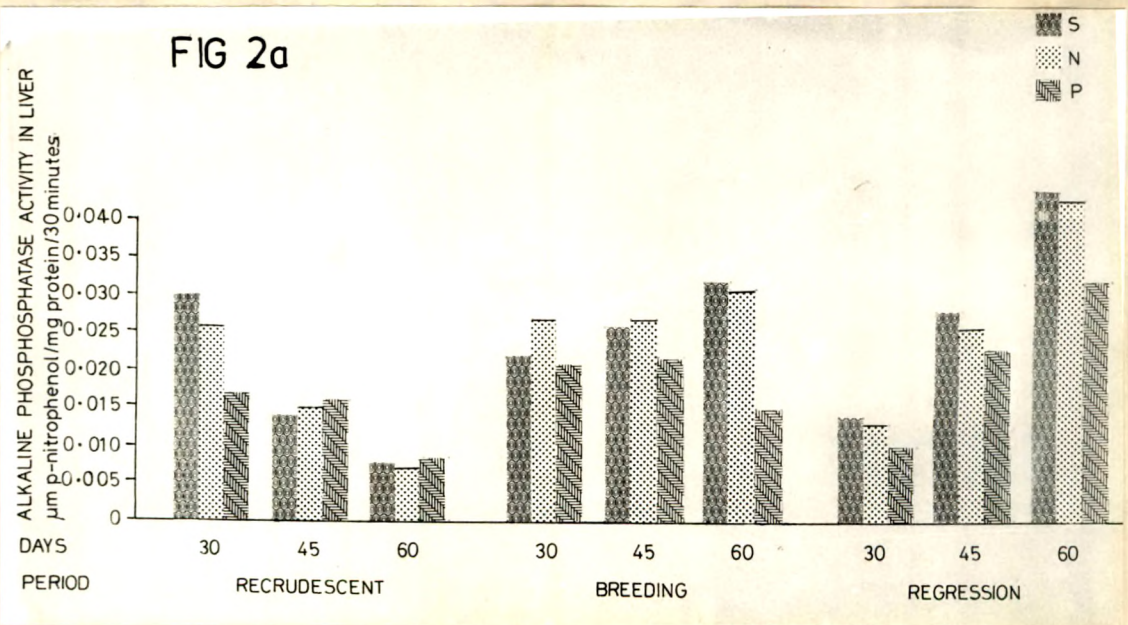

S
N
P
ALKALINE PHOSPHOSPHATASE ACTIVITY IN LIVER
µm p-nitrophenol/mg protein/30 minutes
0·040
0·035
0·030
0·025
0·020
0·015
0·010
0·005
0
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG 2b

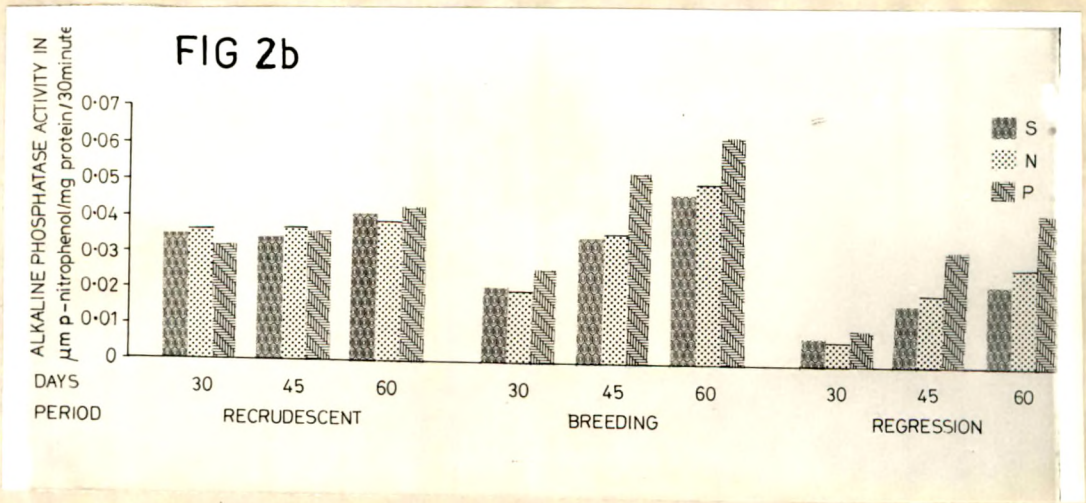

ALKALINE PHOSPHATASE ACTIVITY IN
µm p-nitrophenol/mg protein/30minute
0·07
0·06
0·05
0·04
0·03
0·02
0·01
0
S
N
P
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG 2c

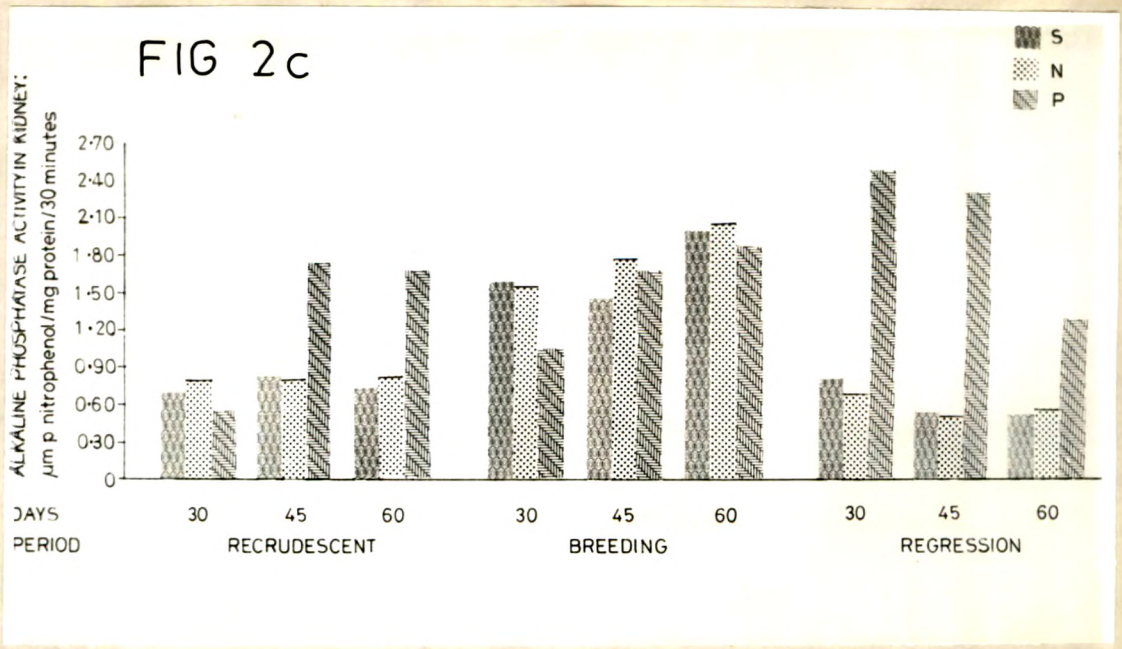

S
N
P
ALKALINE PHOSPHATASE ACTIVITY IN KIDNEY:
µm p nitrophenol/mg protein/30 minutes
2·70
2·40
2·10
1·80
1·50
1·20
0·90
0·60
0·30
0
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG 2d

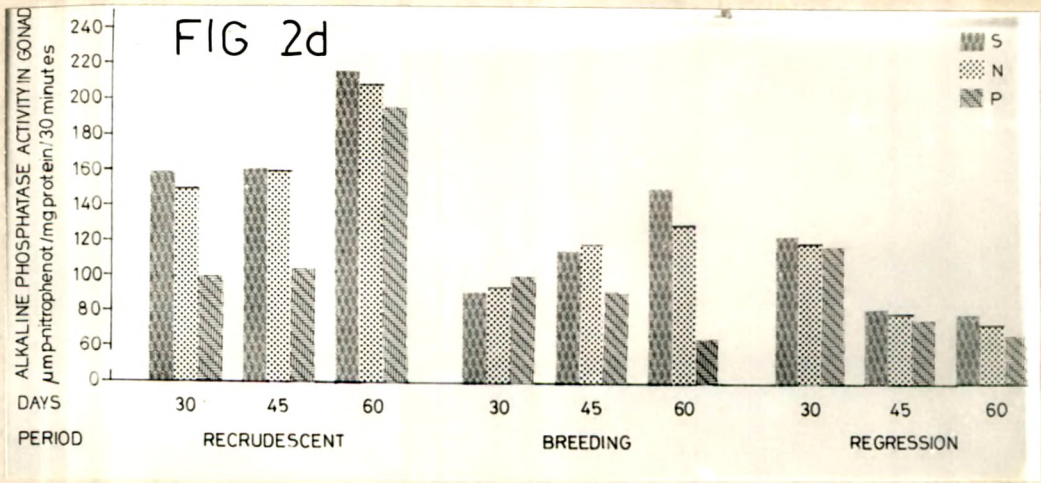

S
N
P
ALKALINE PHOSPHATASE ACTIVITY IN GONAD
µm p-nitrophenol/mg protein/30 minutes
240
220
200
180
160
140
120
100
80
60
0
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG. 3a

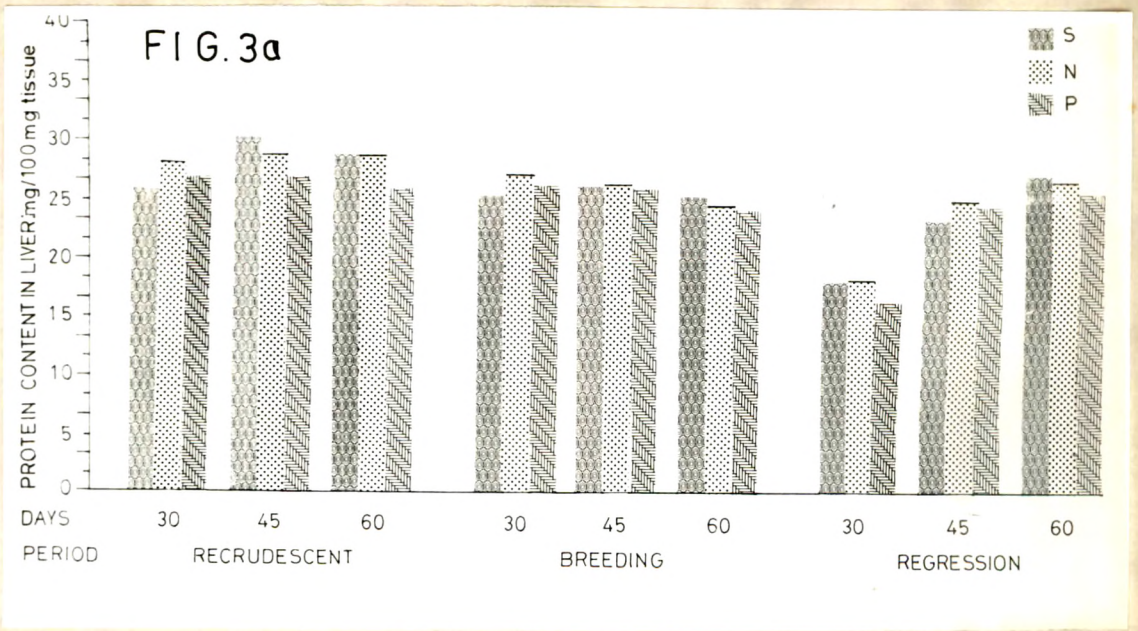

PROTEIN CONTENT IN LIVER:mg/100mg tissue
40
35
30
25
20
15
10
5
0
S
N
P
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG. 3b

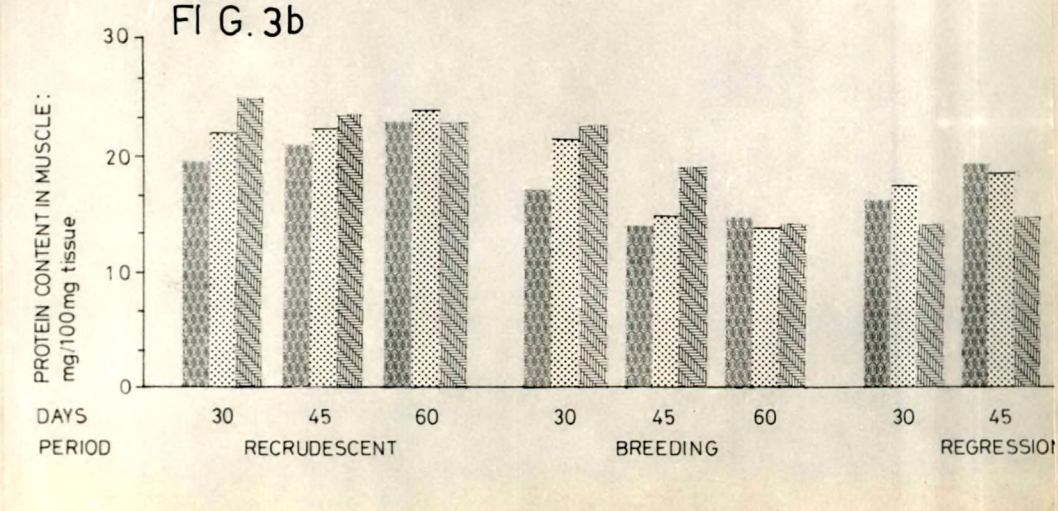

PROTEIN CONTENT IN MUSCLE:
mg/100mg tissue
30
20
10
0
DAYS
30
45
60
30
45
60
30
45
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG. 3c

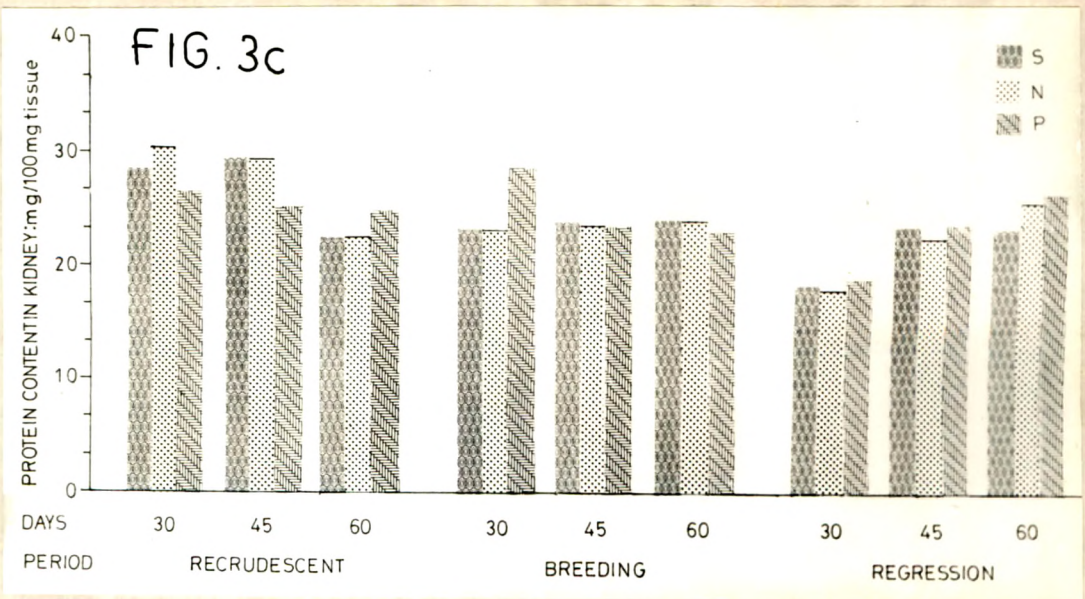
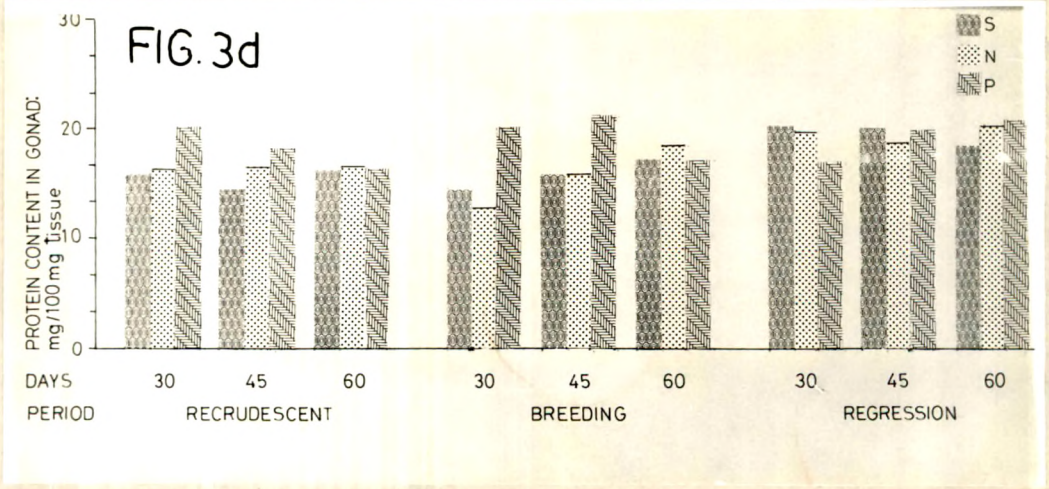

PROTEIN CONTENT IN KIDNEY: mg/100mg tissue
40
30
20
10
0
S
N
P
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG. 3d


30
20
10
0
PROTEIN CONTENT IN GONAD: mg/100mg tissue
S
N
P
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

CHANGES DUE TO PINEALECTOMY:

Gonadal acid phosphatase activity decreased significantly in PX birds during recrudescence and breeding while it increased during regression. The enzyme activity did not show any appreciable change in liver post-pinealectomy, in any of the phases; however PX appeared to have an inhibitory influence on the normal seasonal changes shown by the controls. In muscle, acid phosphatase activity was elevated in PX birds during recrudescence and regression, with almost no change during breeding. Acid phosphatase activity of kidney was increased in recrudescence, decreased in regression and slightly lowered during breeding. PX appeared to have a differential influence on alk. phosphatase activity in the various tissue studied. Whereas PX had a lowering influence on the enzyme activity in liver and gonads during all the phases, it had a positive influence in muscle during breeding and regression, and in kidney during recrudescence and regression. Gonadal and muscle protein contents was increased in PX birds during both recredescence and breeding. In the regression period, whereas the gonadal protein content was unaltered, the muscle protein content was decreased, Protein content of liver had a tendency to decrease during recrudescence and regression, while that of kidney was decreased only during recrudescence, and increased during breeding.

DISCUSSION

Non-specific phosphatases (acid and alkaline) are known to be versatile in their functional association with many biological processes. They are reported to be involved in quite a few spatial and temporal fluctuations in metabolic patterns. Like other animals, annual reproductive cyclicity in aves also calls for tremendous metabolic shifts and adjustments designed to meet the exigencies of gonadal recrudescence and breeding activities. The previous chapters have sufficiently substantiated this aspect. Quantitative alterations in metabolite content and enzyme activity of various organs and tissues characterise such seasonal changes and the presently noted increased activity of acid and alkaline phosphatases and decreasing content of protein in liver, muscle, kidney and gonads during recrudescent and breeding phases are self explanatory. Gonadal awakening and the ensuing breeding activities in wild pigeons appear to have an inverse relationship with protein content of not only gonads, but also other organs such as liver, muscle and kidney. A generalized influence on protein metabolism either in the form of reduced protein anabolism or increased catabolism could be presumed. It is known that in some birds, heralding of breeding activities is marked by reduced thyroid activity (Patel, 1976). In the present study, a progonadal role has been suggested for pineal in feral pigeons. Accordingly, the increasing activity of pineal

during recrudescence was correlated with decreasing thyroid activity (chapter 1) and reduced insulin influence due to the suggested anti-insulinic role of pineal (chapter 3). Both these aspects can have a direct correlation with the present result of decreasing protein content during active reproductive phases, as thyroxine and insulin have both protein anabolic influence. Unlike the protein content, the activity of both the phosphatases show a parallel correlation with gonadal growth and activity in all the organs, once again emphasising a generalised influence. Similar changes in hepatic and renal phosphatases have been reported by Patel (1976) with respect to reproductive activity in Sturnus roseus and Motacilla alba. Such alterations in tissue activity of these non-specific phosphatases could be due to the involvement of these enzymes in various physiological mechanisms characteristic of annual cyclicity and probably influenced by sex steroids.

Whereas pinealectomy induced differential seasonal and tissue specific changes in the activity of phosphatases, a significant uniform alteration in tissue protein content was obtained only in the breeding phase in the form of an increase. In fact, gonadal protein content of PX birds was higher during both recrudescence and breeding. Thus these facts suggest the inability of PX birds to bring about the adaptive alteration in protein content which may have

correlation with the observed shrinkage of their gonads (chapter 1), and probably also indicates the definite involvement of an interaction between pineal principle(s) and one or more other endocrine factors. This is further illustrated by the observed differential alteration in activity levels of the phosphatases. In the absence of clearcut literature regarding the relationship between hormonal factors and phosphatases, it is all the more difficult to visualise the involvement of pineal, either direct or indirect, in season specific regulation of non specific phosphtases. A direct correlation between pineal and gonadal phosphatase activity can however be drawn from the observed decreased activity in PX pigeons of both the phosphatases in the recrudescent and breeding phases. The increased acid phosphatase activity during regression in PX condition is not explainable and the concept of more than one pineal principle with varied influence appears tenable in this context. Apart from the season specific alterations, tissue specific alterations are also discernible as shown by the increased activity of both the enzymes in the muscle of PX birds and of alkaline phosphatase in the kidney during recrudescence. Such temporal and spatial variations in enzyme activity in the absence of pineal though precludes formulating any generalized concept of the involvement of pineal, do nevertheless indicate a definite participation of pineal in the normal homeostatic adjustments and the skewing up of the seasonal adjustments in the absence of

pineal. Like the gonads, the liver also showed reduced alkaline phosphatase activity in PX birds. Modifications in hepatic alkaline phosphatase of mouse following administration of histamine and serotonin have been reported (Thorbecke et al., 1961). The presently observed alteration in hepatic alkaline phosphatase activity in the pinealectomized conditions is whether due to a reduced content of serotonin or histamine is worth ascertaining.