CHAPTER 3

SEASONAL QUANTITATIVE ALTERATIONS IN GLYCOGEN, AND PHOSPHORYLASE ACTIVITY IN LIVER AND MUSCLE OF NORMAL AND PINEALECTOMIZED FERAL BLUE ROCK PIGEON, *COLUMBA LIVIA*.

Most of the vertebrate species show presence of glycogen as the carbohydrate reserve. In birds and mammals, glycogen is found in most tissues of the body of which liver has the richest store. Potential energy locked up in the molecules of glycogen is stored in various tissues for the total body economy. It is readily and rapidly synthesised from all available nutrients and is easily broken down when energy demand of the body increases. Because of its gigantic molecular size, limited solubility and less osmotic effect, glycogen is the preferable storing material in the body. Glycogen plays an important role in maintaining normal blood glucose level in vertebrates.

Insulin and glucagon are hormones involved in regulation of carbohydrate metabolism. In birds, insulin is reported to have only a secondary role in carbohydrate metabolism (Hazelwood, 1973) and hence are primarily glucagon dependent. Mondon and Burton (1971) reported participation of neurotransmitters in sugar transport. Degradation of glycogen is accomplished by the enzyme, phosphorylase. This enzyme is of two types, liver type phosphorylase and muscle type phosphorylase. Both types exist in active as well as inactive forms. [illegible] Glycogenolytic process gives a direct indication of active phosphorylase activity. It is needless to say that energy requirement by different tissues in seasonal birds would vary according to different phases of their reproductive activity. Pineal is reported to have definite influence in reproductive activities of both, birds and mammals. Seasonal reproductive activities involving development and regression of reproductive structures controlled by endocrine factors usually bring about in their wake altered metabolic patterns and modified energy equilibrium. Quantitative levels of metabolites, enzymes <u>etc</u>. are known to undergo adaptive changes during seasonal breeding activities. Since pineal is suspected to have some sort of an axis with reproductive structures, and as no information is available on influence of pineal in general metabolism, it was thought worthwhile to study the effect of pineal ablation on quantitative levels of glycogen, and phosphorylase activity in liver and muscle of feral blue rock pigeon, Columba livia, during recrudescent, breeding and regression phases of its annual reproductive cycle. To have an idea about the temporal effect of pinealectomy on carbohydrate metabolism, three experimental time periods of 30, 45 and 60 days post-pinealectomy were considered in all phases of the annual cycle.

MATERIALS AND METHODS

Adult pigeons were procured from the local animal dealer and maintained in the aviary on grains and water *ad libitum*. Birds were brought into the experimental set-up after allowing at least a fortnight of acclimation. Surgical removal of pineal was done as per the method described in chapter one, and kept in the aviary after taking suitable post-operative care, to be used after 30, 45 and 60 days post-pinealectomy. Corresponding sham-operated pigeons together with normal ones were also kept in the aviary for use at different time periods. In all the three stages of the reproductive cycle *viz.* recrudescence, breeding and regression, pigeons of either sex [8 birds: 4 males and 4 females; control (C), sham control (PN) and experimental (PX) each] were brought from the aviary at the end of each experimental period. Birds were decapitated and a piece of the breast muscle and a piece from the right liver lobe were immediately excised. After blotting the tissues free of tissue fluids *etc.* glycogen was estimated according to the method of Seifter *et al.* (1950). Tissues were digested in 2 ml of hot 30 % KOH and anthrone reagent was used for developing the colour. Optical density was measured at 620 mμ using "Spectronic 20" photoelectric colorimeter and the glycogen content was expressed as percentage of wet tissue weight.

Phosphorylase activity in liver and muscle was assayed by a modification of the method of Cori *et al.* (1943) as adapted by Cahill *et al.* (1957). Glucose-1-phosphate (dipotassium salt, Sigma Chemical Co.) was used as substrate and the inorganic phosphate released was measured according to the method of Fiske and Subbarao (1925). The readings were taken at 660 mμ on Klett Summerson photoelectric colorimeter. Protein content was estimated by the method of Lowry *et al.* (1951) and the enzyme activity expressed as μg of phosphorus released / mg protein / 10 minutes.

RESULTS

Quantitative alterations in glycogen content and phosphorylase activity of liver and muscle of normal (C),

EXPLANATIONS FOR FIGURES

Figures 1 to 4. Histogram showing alterations in glycogen, and phosphorylase activity in liver and muscle in three different phases of reproductive activity, at three different intervals post-pinealectomy.

Fig. 1. Glycogen in liver

Fig. 2. Glycogen in muscle

Fig. 3. Phosphorylase activity in liver

Fig. 4. Phosphorylase activity in muscle

Abbreviations:

S - Sham operated

N - Normal unoperated

P - Pinealectomized

FIG.1

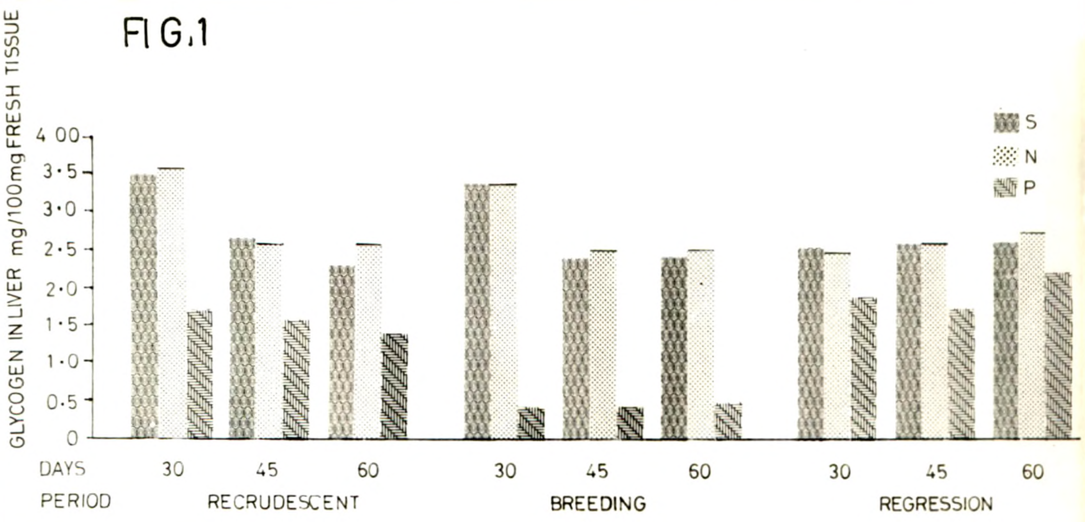

GLYCOGEN IN LIVER mg/100mg FRESH TISSUE
4 00
3·5
3·0
2·5
2·0
1·5
1·0
0·5
0
S
N
P
DAYS 30 45 60 30 45 60 30 45 60
PERIOD RECRUDESCENT BREEDING REGRESSION

FIG.2

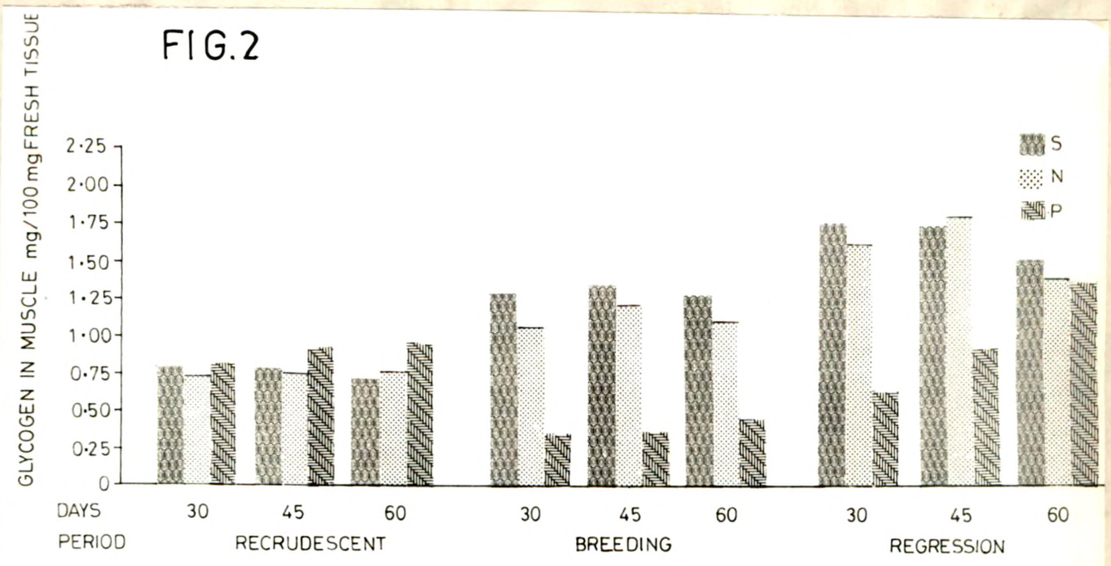

GLYCOGEN IN MUSCLE mg/100 mg FRESH TISSUE
2·25
2·00
1·75
1·50
1·25
1·00
0·75
0·50
0·25
0
S
N
P
DAYS 30 45 60 30 45 60 30 45 60
PERIOD RECRUDESCENT BREEDING REGRESSION

FIG. 3

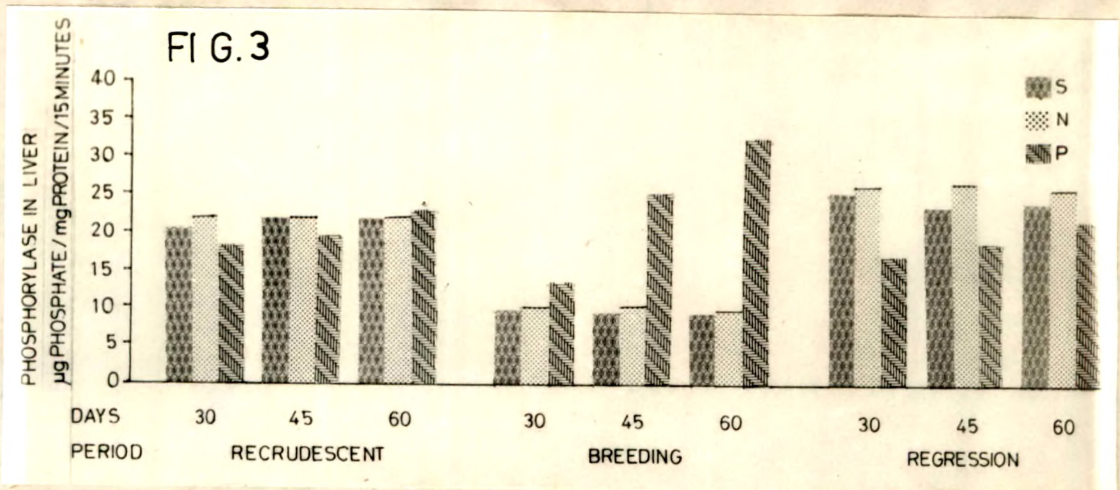
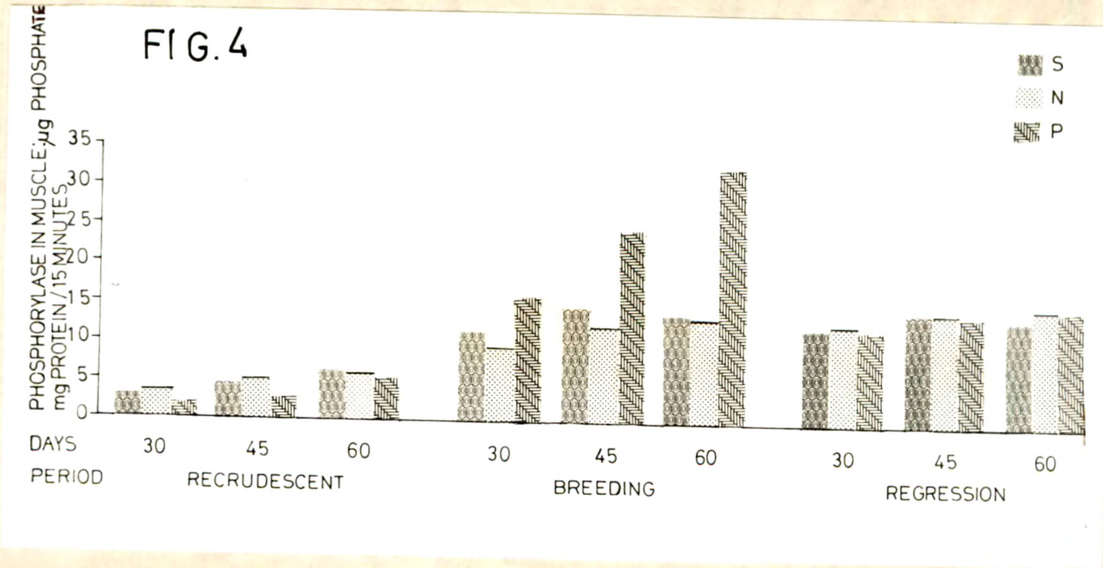

PHOSPHORYLASE IN LIVER
µg PHOSPHATE / mg PROTEIN / 15 MINUTES
40
35
30
25
20
15
10
5
0
S
N
P
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG. 4


PHOSPHORYLASE IN MUSCLE: µg PHOSPHATE
mg PROTEIN / 15 MINUTES
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

sham-operated (PN) and pinealectomized (PX) pigeons during the three reproductive phases are represented in tables 1 , 2 and figures 1 to 4.

SEASONAL CHANGES IN NORMAL PIGEONS:

Glycogen content of liver was maximal during the late recrudescence / early breeding periods and lowest during the early regression period; a reduction by 30 % from the maximal recrudescent level. By late regression, it did show an increase (11 %) which was again lowered slightly by early recrudescent phase (6 %). There was a noticeable increase in hepatic glycogen content during the progressive phases of gonadal recrudescence (38 %). Hepatic phosphorylase activity was in accordance with glycogen; maximal during regression and slightly lower in the recrudescent period. The enzyme activity was, however, much reduced during the breeding months.

In contrast, muscle glycogen content was maximal during regression phase and minimal during recrudescent period. Breeding period showed an intermediate level denoting an active process of glycogen deposition (57 % increase from recrudescence) which reached its maximum in the regression period showing further increase by another 57 %. An interesting observation is the parallel changes in muscle phosphorylase activity (with glycogen) of minimal, intermediate and maximal levels during recrudescent, breeding and regression periods respectively.

CHANGES DUE TO PINEALECTOMY:

Pinealectomy in general led to a lowering of glycogen content in both the liver and muscle. In the case of liver the decrease was maximal during the breeding and recrudescent periods in that order and minimal during regression period. However, muscle glycogen was reduced maximally during breeding and early - mid regression, while it was only marginal during late regression. Contrastingly, muscle glycogen showed an increase during recrudescent period. Phosphorylase activity in both liver and muscle was increased to above normal levels only during the breeding periods; with below normal levels being recorded during recrudescent and regression periods by hepatic phosphorylase. Muscle phosphorylase on the other hand did show a slightly reduced level during recrudescence and no alteration in the regression phase. An interesting observation is the general tendency for temporal increase in phosphorylase activity with increasing time periods post-pinealectomy in all the breeding phases.

DISCUSSION

Information on seasonal alterations in carbohydrate metabolism in birds in general, and wild species in particular, is not adequate. Even hormonal regulation of carbohydrate metabolism in the avian species is not that well documented as compared to mammals. In the present study, definite seasonal modulations in the glycogen store of liver and muscle have been evinced. On a comparative basis, it is clear that muscle glycogen store has depicted more significant changes than the liver. A depletion of muscle glycogen to the tune of about 60 % during recrudescence through late regression justifies considering the muscle glycogen store as an important energy source during gonadal recrudescence in wild pigeons. Breeding period appears to be an active phase of glycogenesis in muscle, and the apparently limited requirement of carbohydrates is met by the hepatic tissue which shows a 20 % depletion at this time. Since the changes in phosphorylase activity are parallel with that of glycogen in both liver and muscle, obviously the enzyme activity is modulated parallel to that of the metabolite load.

Pineal ablation led to a decreased glycogen content in both the organs studied, except for the muscle in the recrudescent period, whose content was elevated. Obviously, hepatic glycogen depletion was maximal during breeding and recrudescent periods as compared to the regression period. Delahunty *et al.* (1980) have in their study on pineal in carbohydrate metabolism in the fish, *Carassius auratus*, observed lowered hepatic glycogen levels in pinealectomized fishes in the month of May, while no alteration occured in the month of January. These observations tend to indicate a season specific pineal involvement in carbohydrate metabolism of vertebrates. Highly elevated phosphorylase activity in the pinealectomized birds during the breeding months corresponding to the much lowered glycogen stores of liver and muscle might call for an altered hormonal set-up leading to excessive phosphorylase activation. Some idea about the involvement of gonadal hormones in controlling [illegible] hepatic phosphorylase activity can be gained by the reported increase in phosphorylase activity, post-castration in rats, and its decrease by replacement therapy (Ambadkar and Gangaramani, 1981). In this light, the increased phosphorylase activity obtained in the present study, might in all probability represent the effect of a reduced titre of gonadal steroids post-pineal ablation. This is based on the purported progonadal nature of pineal in pigeon as observed by the gonadal regression after the extirpation of the gland (chapter 1). Further, the sub-normal levels of hepatic and muscle phosphorylase noticeable during recrudescent and regression periods, and the increased muscle glycogen content recorded in the recrudescent period also highlight some intriguing altered seasonal interactions between various endocrine factors in the absence of pineal principles. A correlation between phosphorylase and pineal is also tentatively surmisable by the depicted tendency of temporal increase in enzyme activity with increasing time periods post-pinealectomy in all the phases of reproduction. Some inexplicable direct or indirect effect on the genetic locus of phosphorylase seems a possibility in this wake. It is also pertinent that, since the levels of phosphorylase and its substrate (glycogen) are not strictly correlatory in the expected opposite fashion, either in the control or in the pinealectomized birds, a clear distinction between total quantitative content of the enzyme and its active form existing at any moment needs to be drawn, and as such are apparently different.

Finally, in the light of the suggested relation of pineal and pancreas (Gorray et al., 1979; Mihail and Giurgea, 1979; Mihail et al., 1980), and the currently observed glycogen lowering effect as well as the hypoglycemic tendency in pinealectomized birds (chapter 2), the probable anti-insulinic and anti-avian pancreatic polypeptide (APP) role of pineal in the wild pigeons need to be explored. This is pertinent in view of the influence of APP in lowering hepatic glycogen without bringing about hyperglycemia and that of insulin in lowering glucose level (Hazelwood et al., 1973; Hazelwood,1978; Hazelwood and Langslow, 1978).