

CHAPTER 4

SEASONAL ALTERATIONS IN ADENOSINE TRIPHOSPHATASE,
SUCCINATE-DEHYDROGENASE AND GLUCOSE-6-PHOSPHATASE
ACTIVITY IN LIVER, MUSCLE, KIDNEY AND GONADS OF
NORMAL AND PINEALECTOMIZED FERAL BLUE ROCK PIGEON,
COLUMBA LIVIA.

In accordance with functional and non-functional states of gonads in seasonally breeding birds and mammals, well defined histophysiological and biochemical changes in response to hormonal factors in various tissues have been reported by many workers. Recently, studies on histophysiology of many seasonally breeding birds have been documented (Chalana and Guraya, 1974, 1977, 1978 ; Ambadkar and Chauhan, 1976; Mori and George, 1978). Garnier et al. (1973) have reported hormonal changes in testicular cycles of the pekin duck. Effects of pineal removal and its influence on prolactin and sex steroids were also studied in rats (Takahashi et al., 1979). Recent works have demonstrated the involvement of pineal in the control of

reproduction of some mammals (Wurtman et al., 1968) and also in the seasonally breeding Indian weaver bird (Saxena et al., 1978).

Most of the studies carried out on avian and mammalian pineal gland were directed exclusively to its influence on reproductive organs. Recently, some workers have made an attempt to study the role of pineal gland in carbohydrate metabolism in rat (Hirschmann and Stahl, 1979). No such studies are known to have been done in any avian species. Adenosine-tri-phosphatase (ATPase) is actively involved in high energy phosphate metabolism. Variations in activity of ATPase in different tissues have been observed with respect to the concentration of Mg^{++} and Ca^{++} ions, types of inhibitors, activators, pH optima, substrate concentration etc. Mg^{++} dependent ATPase is considered to be universally present in all animal tissues. Succinate dehydrogenase (SDH) is a key enzyme of Krebs cycle which usually functions as an index of oxidative metabolism. Glucose-6-phosphatase (G6Pase) is an important enzyme in the intermediary metabolism. From the activities of these enzymes some information on the relative occurrence of gluconeogenesis, oxidative metabolism and ATP utilization can be gained. To get a generalized idea regarding the role of pineal gland on intermediary metabolism, present study on quantitative analysis of ATPase, SDH and G6Pase

was undertaken during various phases of the seasonal reproductive cycle of the feral blue rock pigeon, Columba livia.

MATERIALS AND METHODS

Normal healthy pigeons of both sexes were obtained from the local animal dealer and were acclimated to the laboratory conditions for a fortnight. Pinealectomy was performed according to the procedure described in chapter 1 and were then housed in an aviary providing water and food ad libitum. At the end of each experimental periods (i.e. 30, 45 and 60 days post-pinealectomy and during each reproductive phase) pigeons (C, PN, PX conditions) were brought from the aviary. They were weighed and sacrificed by decapitation. Slices of breast muscle, right liver lobe, a lobe of the kidney and gonad were cut quickly and blotted free of blood and tissue fluids. Tissue homogenates prepared in chilled distilled water were used for quantitative analysis of adenosine triphosphatase and succinate dehydrogenase.

Liver and muscle adenosine triphosphatase activity (ATPase - Mg^{++} activated) was measured quantitatively adopting the method of Umbreit et al. (1957). Adenosine-5-

triphosphate (disodium salt, Sigma Chemicals Co., U.S.A.) was used as the substrate. Inorganic phosphate released was estimated according to the method of Fiske and Subbarao (1925). The readings were taken at 660μ on a Klett-Summerson photoelectric colorimeter. The enzyme activity was expressed as μg of phosphorus released / mg protein / 10 minutes.

Succinate dehydrogenase activity was assayed in the liver, muscle and kidney homogenates according to the method of Kun and Abood (1949). Triphenyl tetrazolium chloride (TTC) was used as the hydrogen acceptor. The enzyme activity was read at 420μ on a Klett-Summerson photoelectric colorimeter. Protein was estimated employing the method of Lowry et al. (1951). The enzyme activity was expressed as μg formazan formed / mg protein / 30 minutes.

Homogenates of liver and gonads for glucose-6-phosphatase assay were prepared in cold citrate buffer at pH 6.5 and the method of Harper (1963) was employed. Glucose-6-phosphate (disodium salt) was used as the substrate. Inorganic phosphate released was measured employing the method of Fiske and Subbarao (1925). The readings were taken at 660μ on a Klett-Summerson colorimeter. Activity of the enzyme was expressed as μg phosphorus released / mg protein / 15 minutes.

RESULTS

ATPase, SDH and G6Pase activities in different tissues are represented in tables 1,2,3 and figures 1,2 and 3 respectively.

SEASONAL CHANGES IN NORMAL BIRDS:

SDH and ATPase activities tended to be more or less of a reverse pattern in liver and muscle during the three reproductive phases. Accordingly, hepatic ATPase activity increased during the recrudescence period and showed a continuous decrease from breeding through regression. Correspondingly, hepatic SDH activity decreased during recrudescence, remained steady at an elevated level during the breeding and started increasing during regression after a post-breeding fall. In contrast, muscle ATPase activity decreased during recrudescence, remained at an increased level during breeding and then showed a decrease during regression. Muscle SDH activity on the other hand increased during recrudescence, showed a more pronounced increase during breeding and then remained steady at a low level during regression. Kidney SDH activity showed maximal activity at the late recrudescence from which it decreased continuously during breeding to reach the lowest level at the early regression phase. Since then, kidney SDH activity increased continuously through late regression and recrudescence periods. G6Pase activity of both liver and gonads showed an increasing trend during recrudescence, elevated levels during breeding and again an increasing trend during regression from the reduced levels post-breeding.

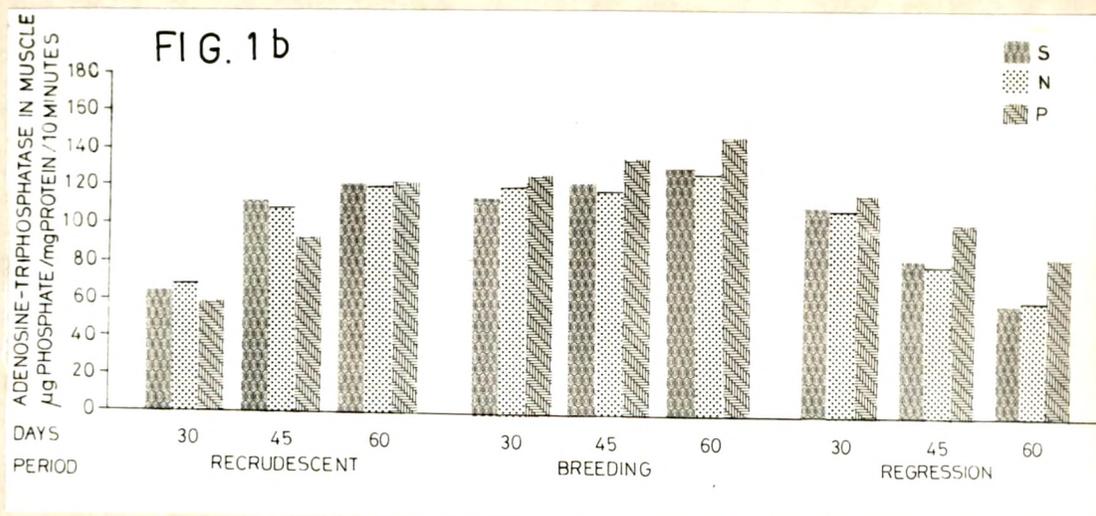
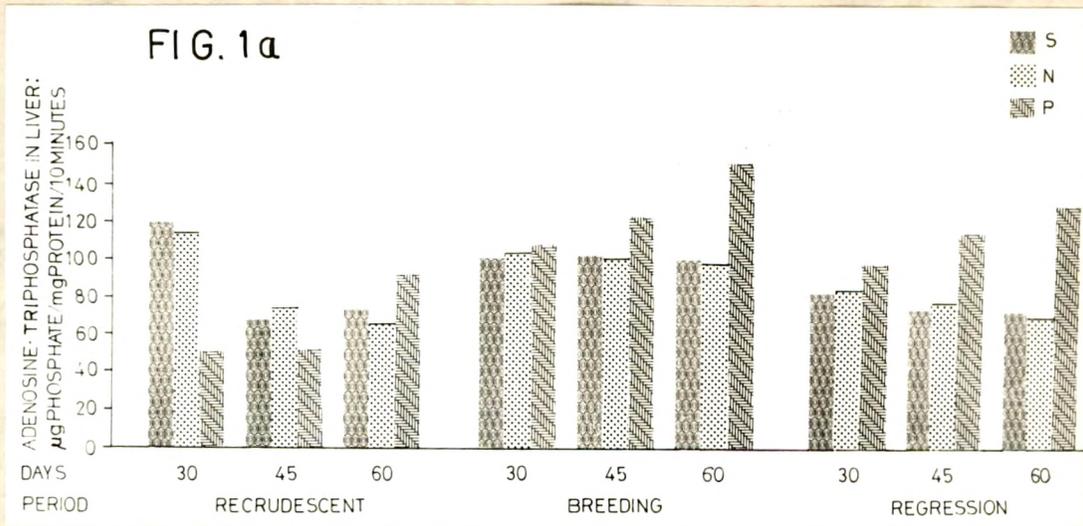
EXPLANATIONS FOR FIGURES

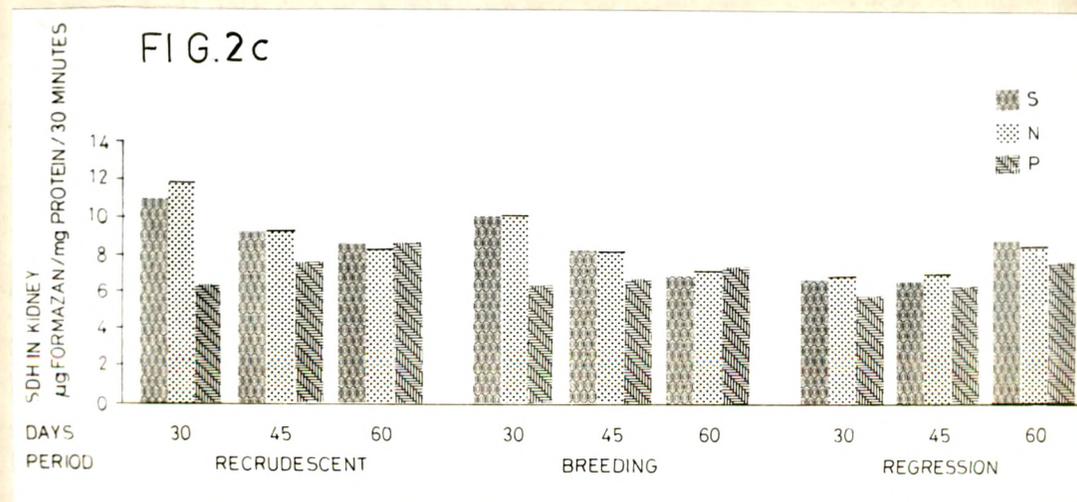
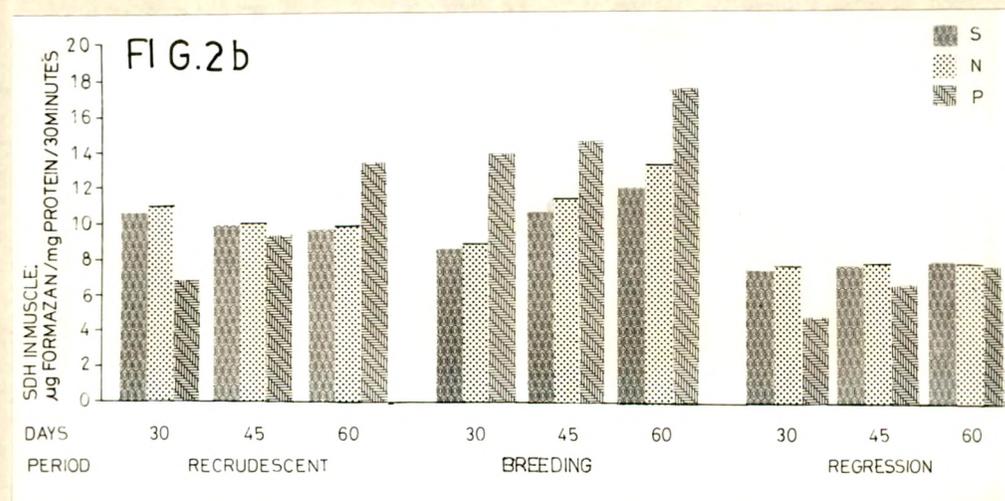
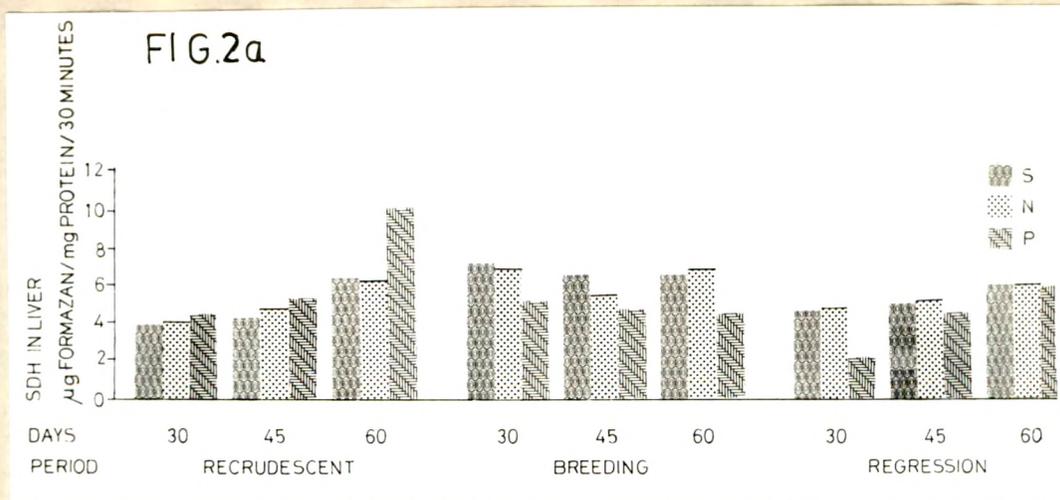
Figs. 1 to 3 : Histograms showing alterations in adenosine triphosphatase, succinate dehydrogenase and glucose-6-phosphatase in liver, muscle, kidney and gonads in three different intervals post-pinealectomy.

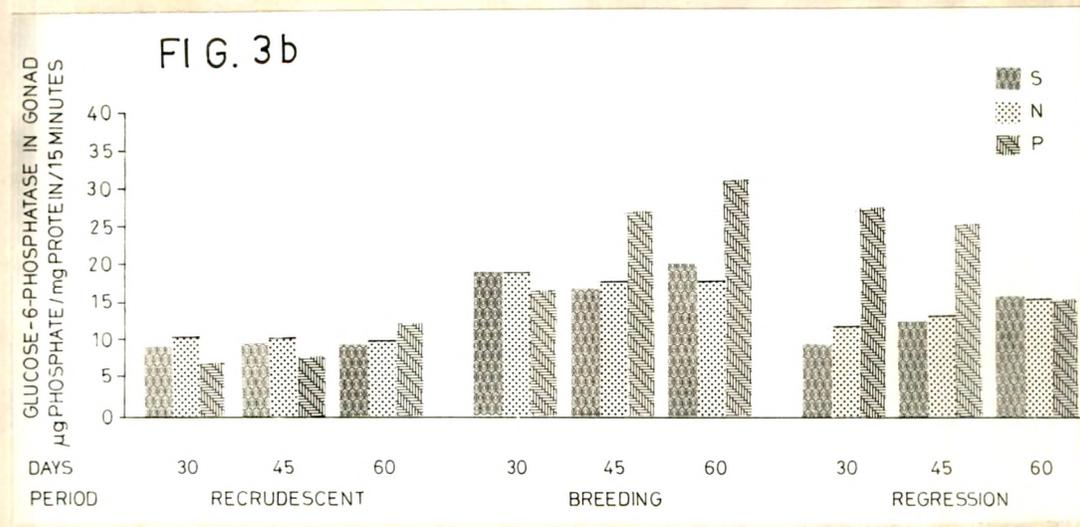
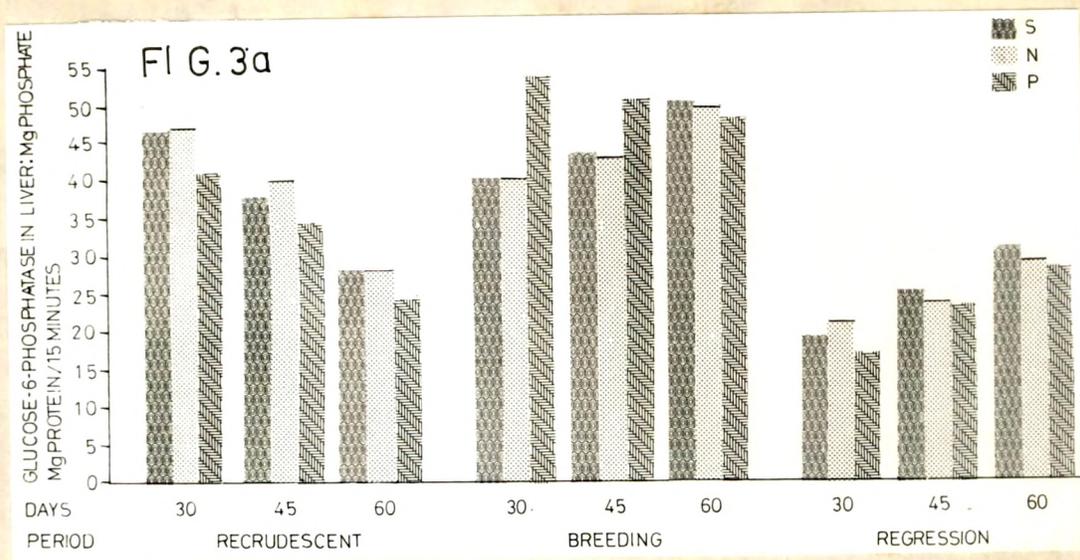
- Fig. 1a - ATPase in liver
- Fig. 1b - ATPase in muscle
- Fig. 2a - SDH in liver
- Fig. 2b - SDH in muscle
- Fig. 2c - SDH in kidney
- Fig. 3a - G-6-Pase in liver
- Fig. 3b - G-6-Pase in gonad

Abbreviations:

- S - Sham operated
- N - Normal unoperated
- P - Pinealectomized







Though the pattern of changes were essentially similar in both the cases, hepatic G6Pase activity showed the lowest levels during regression, while the lowest levels of G6Pase activity in gonads were registered during recrudescence.

CHANGES DUE TO PINEALECTOMY:

In general, hepatic and muscle ATPase showed significant increase in PX birds during both breeding and regression while it was decreased during recrudescence. SDH showed a differential response in the three organs of PX birds. Whereas liver SDH was increased during recrudescence and decreased during breeding and regression, muscle SDH was increased during breeding and decreased during both regression and recrudescence. In contrast, kidney SDH in general tended to show reduction in activity during all the three phases in PX birds. G6Pase activity depicted post-pinelectomy increase in liver during breeding and fall during recrudescence and regression. However, gonad G6Pase tended to show increase in general during breeding and regression, and reduction only during early and mid recrudescence. The various changes outlined above are represented in tables 1,2,3 and figures 1,2,3.

DISCUSSION

ATPase and SDH are actively involved in cell metabolism; the former generally drives catabolic and energy utilizing reactions, while the latter by its active involvement in TCA cycle oxidation keeps up replenishment and supply of energy rich substrate ATP molecules for the former. In the annual reproductive cyclicality of wild pigeons, recrudescence and breeding periods appear to be metabolically and energetically more active as increased SDH and ATPase activities are recorded during the present study in liver, muscle and kidney. Such increased enzyme activities could denote a generalised increase in body metabolism and activity in conjunction with reproductive functions. Increase in activity of enzymes is documented to occur in other avian species, especially in the migratory ones during their pre-migratory phase, prior to migration to their breeding grounds (Patel et al., 1976). Hepatic SDH activity is known to be induced by thyroxine (Moher, 1964; Wolf and Wolf, 1964; Hulbert et al., 1976). The decrease in hepatic SDH activity during recrudescence (figures 2; table 2) could be due to a decreased thyroid output necessitated by its known antigonadal influence in some avian species. Increased SDH activity in the muscle during recrudescence tends to denote higher incidence of oxidative metabolism with concomitant synthesis and storage of ATP molecules,

while the increased ATPase and SDH activities in muscle during breeding mark the utilization of ATP molecules for the probable increased muscular activity associated with breeding. Recrudescence is known to be associated with dramatic changes in lipid content of the gonads in birds (Lofts, 1978).

Significant alterations in the activity levels of SDH and ATPase in the various organs of PX birds during all the three reproductive phases favour the conclusion that the absence of pineal induces an upset in adaptive enzyme regulation. The differential pattern of changes in enzyme activity observable during the three phases of gonadal cycle further indicates that the pineal participates in regulating enzyme activity by its differential mode of interactions with other principles and factors, which varies with organ and season. Increased ATPase activity during breeding post-pinelectomy in various organs (table 1; figure 1), could lead to unwarranted energy utilization and a net negative energy balance. While decreased ATPase activity as during the recrudescence period would in turn result in sub-optimal energy utilization in PX condition. Similarly, alteration in SDH activity can also result in disruptive metabolic activities.

With respect to G6Pase activity, both liver and gonads have shown definite alterations in PX birds from the normal controls during all the three phases. The average levels of G6Pase activity calculated for both liver and gonads for the three reproductive phases show above control levels during breeding, and below control levels during recrudescence in the PX birds (table 3). During the regression phase, significant change in the enzyme activity was shown only by the gonad in the form of an increase. Obviously, pineal ablation does lead to alterations in the G6Pase regulating system. Quite a few factors, such as alterations in sympathetic activity (Suda, 1966), glucose levels (Pesch and Topper, 1963), glycogen content (Pesch and Topper, 1963) etc. could all play pivotal roles in modifying the regulatory system of G6Pase activity in the pinealectomized condition. The present study thus sheds some light on the possible involvement of pineal principles in adaptive interactions in enzyme regulations and thus in overall metabolic strategy of birds.