CHAPTER 1

OBSERVATIONS ON SEASONAL CHANGES IN BODY AND ORGAN WEIGHTS IN NORMAL AND PINEALECTOMIZED FERAL BLUE ROCK PIGEON, *COLUMBA LIVIA*.

The pineal gland is perhaps the most recent organ to have been rediscovered and subjected to exciting investigations. There is now considerable documentation to show that the avian and mammalian pineal participate in the affairs of the gonads, the thyroid, the brain and several other organs and organ systems. Shellabarger (1953) and Kitay and Altschule (1954) claimed that hypertrophy of the fowl's gonads occured following pinealectomy, and that administration of pineal extract brought about gonadal atrophy. Relatively low doses of melatonin reportedly lower rat ovary weight (Wurtman *et al.*, 1963; Narang *et al.*, 1967; Motta *et al.*, 1967). Effect of light and pinealectomy on gonadal maturation and functioning during developmental stages and in adult mammals have been reported by many

workers. According to Wurtman et al. (1961), effects of light and pinealectomy are not additive. Absence of light causes retardation of sexual development in male and female rats; and a similar decrease in the size of the adult reproductive organs was observed by Reiter (1968), with pinealectomy counteracting these effects. Singh and Turner (1967) have demonstrated that melatonin can decrease the weight of gonads of developing chicken while Sayler and Wolfson (1967 ; 1968) showed that pineal stimulates gonadal maturation in quail. Considerable evidences suggest that pineal modifies gonadal growth and function in mammals and birds which appears to vary with the species, age and sex of the experimental animal and the lighting schedules in its environment. It appears that the various pineal indoles and factors play a secondary role in controlling reproduction in both mammals and birds. Studies indicate that the pineal action on reproductive activities is probably mediated via other endocrine hormones (Reiter, 1973; Moszawaska et al., 1975; Quay, 1974). Very little information is available on the possible role of pineal in thyroid and adrenal functioning and most of those available have been reported in mammals. Presently, knowledge is inadequate to draw any conclusion as to whether avian pineal gland participates in the normal function and growth of thyroid and adrenal. Mihail et al. (1980) have made an attempt to study the influence of pineal on endocrine

pancreas in domestic pigeon. All these investigations (as evident) were conducted either on mammals or on domestic species of birds. Moreover, from the number of published data with many controversies, it is very difficult to make any tenable conclusion/s regarding the role of pineal in growth and/or functioning of other endocrine and non-endocrine organs. Further, as far as the available literature is concerned, no work has ever been carried out regarding the involvement of pineal on normal anatomy or growth of spleen and uropygeal gland in any vertebrate species. Domestication is known to modify or eliminate entirely the influence of environment on reproduction. Moreover, influence of pineal on reproduction is considered to depend directly or indirectly on photoperiods. Most of the published literature concerning the effect of photoperiods on pineal gland is based on the experiments conducted in artificial illumination and that too for short durations. An attempt is made here to find out whether pineal ablation has any influence on the anatomy and/or growth of spleen, pancreas, adrenals, thyroid, gonads and uropygeal gland, besides body weights in relation to cyclic seasonal reproductive activity under natural photoperiod regimes with normal environmental factors in the wild species of feral blue rock pigeon, Columba livia.

MATERIALS AND METHODS

Fully grown healthy wild pigeons were procured from the local animal dealer and were kept in an aviary at least for a fortnight under natural photoperiod regimes for acclimation before they were used for experimental work. Both males and females were utilized for the study. Normal healthy adult birds were brought from the aviary and their body weight noted down. Pigeons were anaesthetized with solvent ether and the dorsal head region was defeathered. The exposed skin was cleaned with ethanol and a 2 cm. long skin incision was made mid-sagittaly. A rhomboid shaped window was made in the skull bone with a surgical blade. Pineal along with its stalk was removed with the help of fine forceps. The cut out piece of bone was replaced back in position, the crevices filled with plaster of paris and the skin incision sutured. An antiseptic ointment was applied over the sutured part and the operated birds were kept isolated in separate cages for a few hours. After that they were housed in the aviary of 12' x 12' x 12' size. Removed pineal was observed under a microscope to confirm its complete removal along with the stalk. Sham-operation was performed according to the same procedure described above, except for not removing the pineal. Normal birds were kept with operated birds in the same aviary under natural environmental conditions and served

as the normal controls. All birds in the aviary were fed on a diet of grains and was provided with water ad libitum throughout the experimental period. For the study, the birds were divided into three major groups.

I : Normal unoperated birds treated as controls,(C).

II : Sham-operated birds treated as operated controls, (PN).

III : Pinealectomized birds, (PX).

Group II and III were further divided into subgroups.

PN30 : 30 days post sham-operation group.

PN45 : 45 days post sham-operation group.

PN60 : 60 days post sham-operation group.

PX30 : 30 days post-pinealectomy group.

PX45 : 45 days post-pinealectomy group.

PX60 : 60 days post-pinealectomy group.

Birds of group II and III were sacrificied at the end of their respective experimental periods. Normal birds unoperated (C) as controls were also sacrificed alongwith each experimental group. For experimental investigations, birds were sacrificed during the three stages of reproductive activity: i) Recrudescent phase, (February to mid March.). ii) Breeding phase (late April to early June).

iii) Regression phase (August to early September). Operations were performed for each experimental group in each phase depending on the time of sacrifice. During the recrudescence, PX60, PX45 and PX30 birds were operated and sacrificed with their corresponding PN and C groups in that order whereas, during the breeding and regression phases PX30, PX45 and PX60 groups were used in that order for experimentation.

From each experimental group, wild feral pigeons (4 males and 4 females) were brought from the aviary at the end of each experimental tenure. The birds were weighed and then sacrificed by decapitation. Birds of all the three groups (C, PN, PX) were weighed at the time of killing as well as prior to sham-operation and pinealectomy respectively. Alongwith the PN and PX birds which were weighed prior to operation, normal (C) birds were also weighed. It was always confirmed that there was no sign of the pineal gland by examining the state of the brain of the PX birds. The entire spleen, pancreas, adrenals, thyroid,gonads and uropygeal gland were carefully dissected out, blotted free of body fluids and then weighed on the scheduled day. The organ weights were expressed in mg.

RESULTS

Changes in weights of various organs of normal and pinealectomized birds are represented in table 1 and figure 1 to b.

SEASONAL VARIATIONS IN NORMAL BIRDS:

During the recrudescent period, spleen, adrenal, uropygeal gland and gonads gained weight gradually. In the breeding phase a reduction in weights of these organs was observed, except for gonads which showed almost same weight in the late recrudescent and early breeding phases. Spleen weight reduced drastically in this season. All the three organs depicted a pronounced increase between late breeding and early regression thus registering increased weights at this stage. Since then, there was a progressive fall in weight of all the three during the regression phase. The peak weight of adrenals and thyroid was observed during the early regression period. In general weight of pancreas was more or less constant during all the three phases of the reproductive cycle. However, higher weights were obtained at the beginning of all the three phases followed by progressive reduction in weights. The highest weight for pancreas was observed at the initial part of the breeding phase and lowest was at the end of the regression phase. Thyroid size was maximum during the regression phase and minimum during the breeding period.

CHANGES DUE TO PINEALECTOMY :

At all phases of reproductive activity, pineal

EXPLANATIONS FOR FIGURES

Figs. 1 to 6 : Histogrames showing weight changes of some organs in three different phases of reproductive activity, at three different intervals post-pinealectomy.

Fig.1. Spleen

Fig.2. Pancreas

Fig.3. Adrenal gland

Fig.4. Thyroid gland

Fig.5. Gonad

Fig.6. Uropygeal gland

Abbreviations:

S - Sham-operated.

N - Normal unoperated.

P - Pinealectomized

FIG. 1

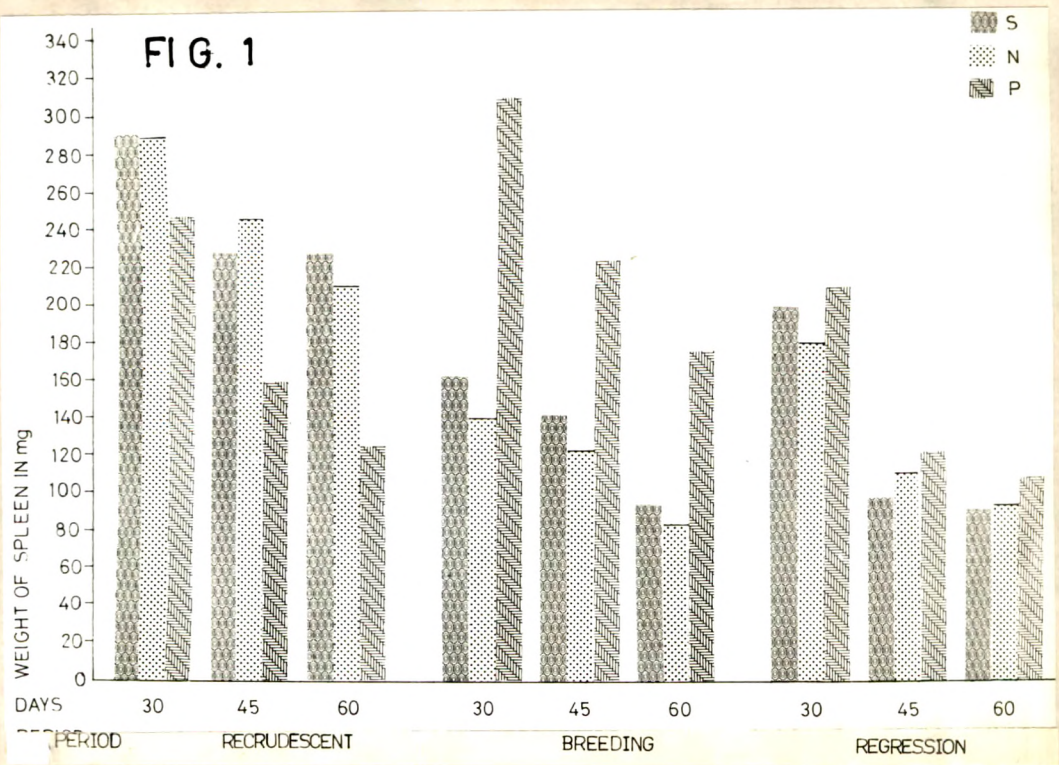

S
N
P
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
WEIGHT OF SPLEEN IN mg
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG.2

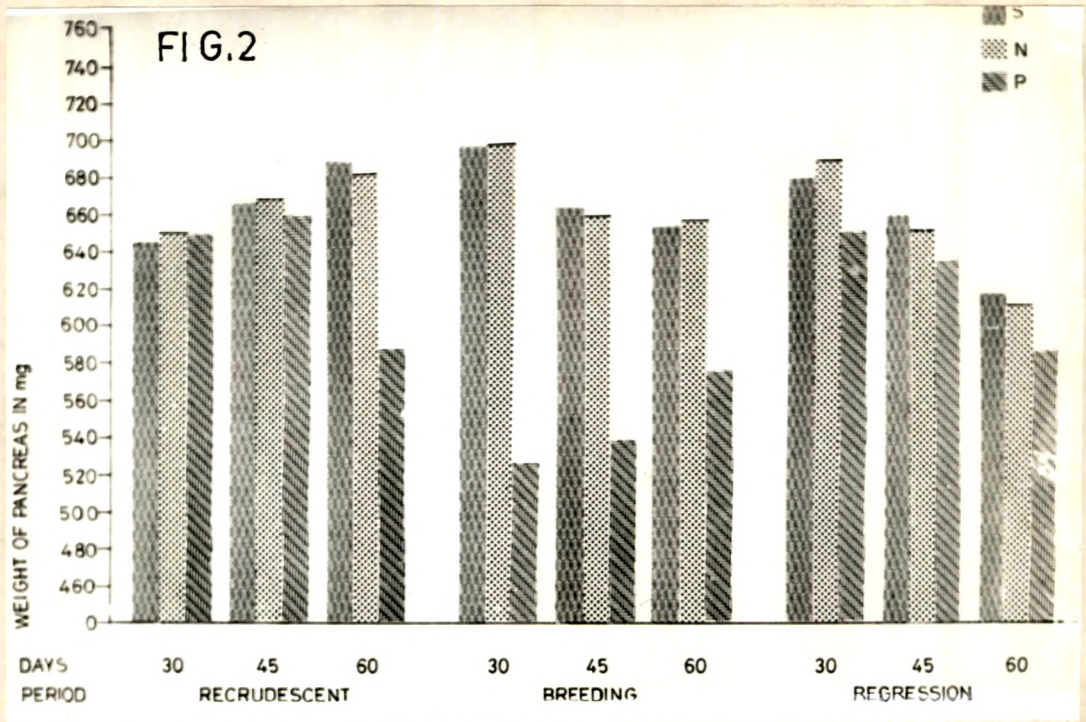

S
N
P
760
740
720
700
680
660
640
620
600
580
560
540
520
500
480
460
0
WEIGHT OF PANCREAS IN mg
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG. 3

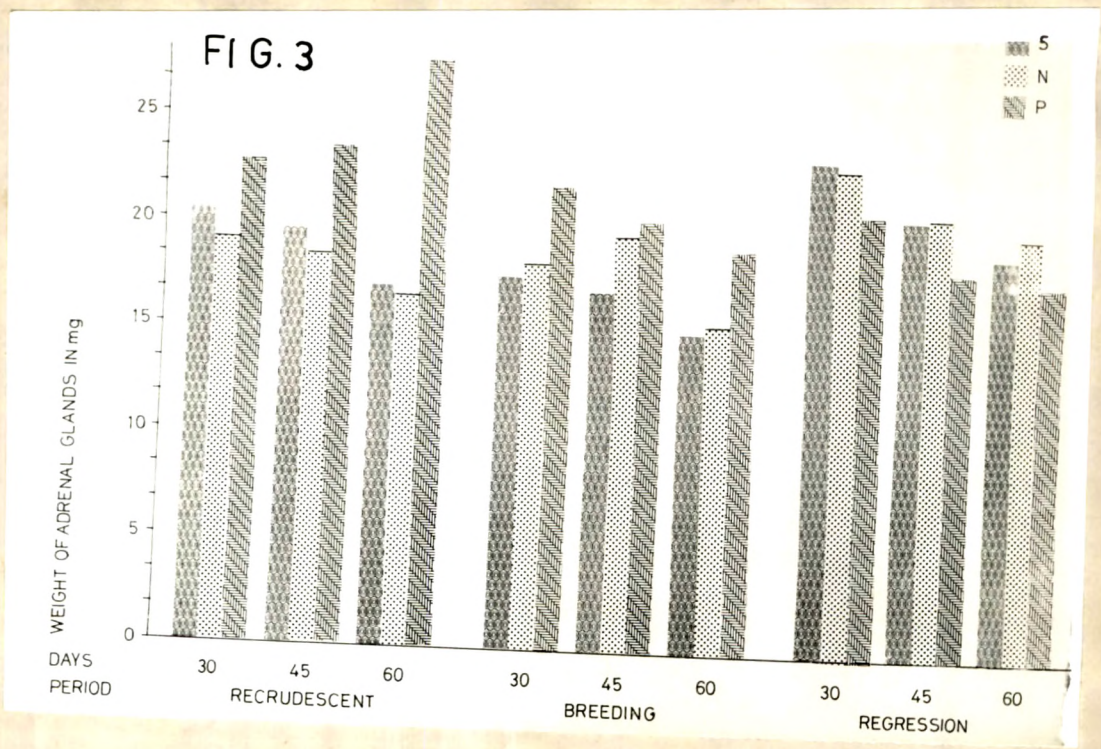
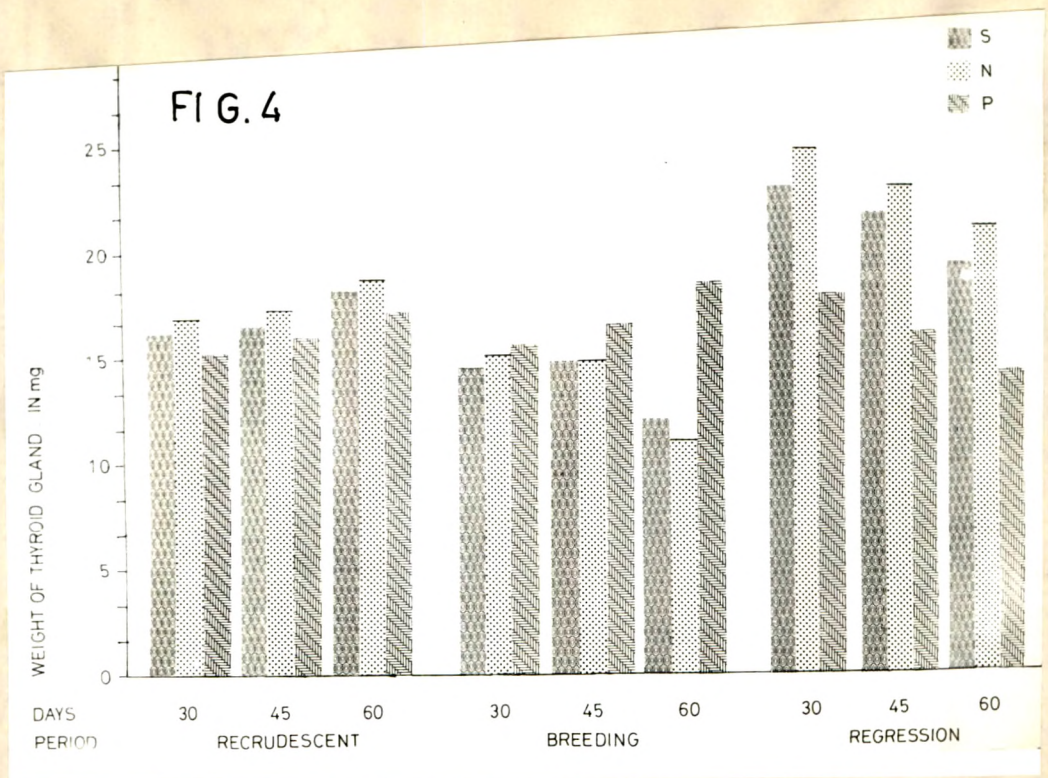

S
N
P
WEIGHT OF ADRENAL GLANDS IN mg
0
5
10
15
20
25
DAYS
PERIOD
30
45
60
RECRUDESCENT
30
45
60
BREEDING
30
45
60
REGRESSION

FIG. 4


S
N
P
WEIGHT OF THYROID GLAND IN mg
0
5
10
15
20
25
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

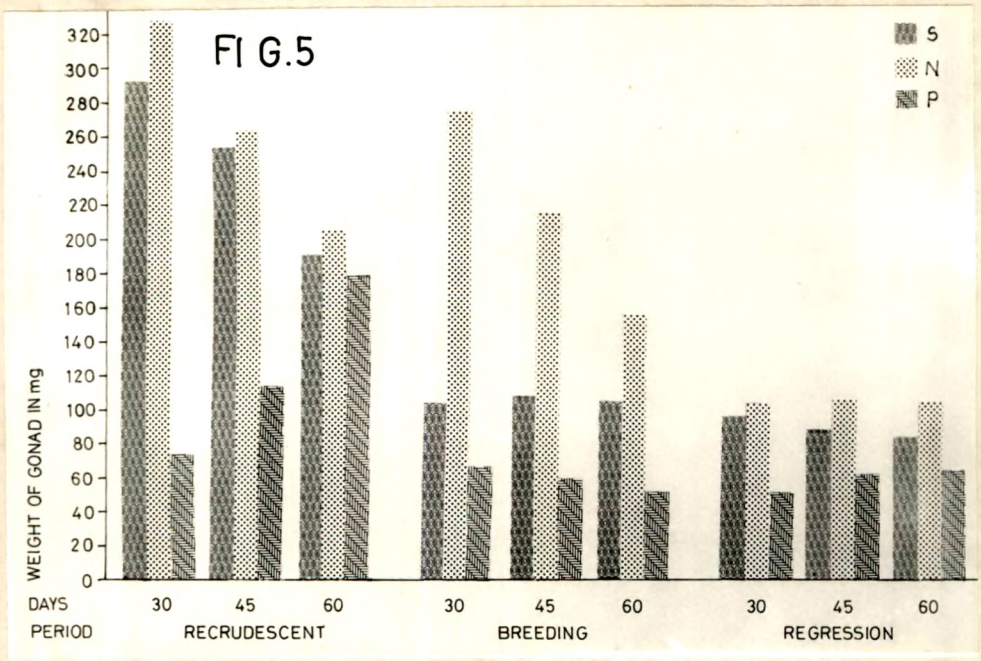
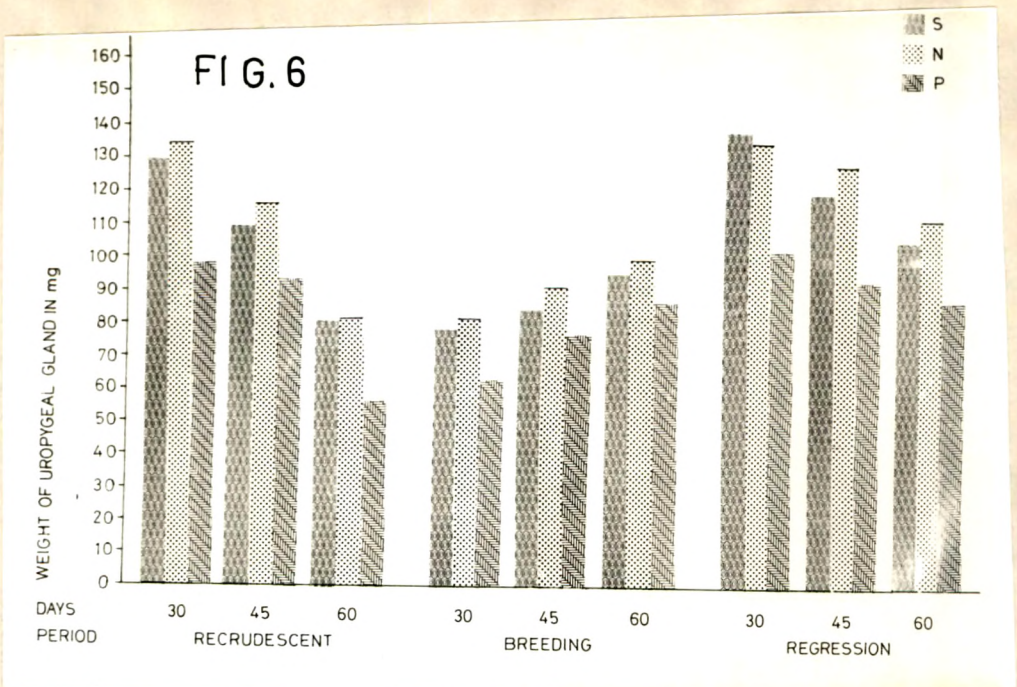


FIG.5
S
N
P
WEIGHT OF GONAD IN mg
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
DAYS
30
45
60
30
45
60
30
45
60
PERIOD
RECRUDESCENT
BREEDING
REGRESSION

FIG. 6


S
N
P
WEIGHT OF UROPYGEAL GLAND IN mg
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
DAYS
PERIOD
30
45
60
RECRUDESCENT
30
45
60
BREEDING
30
45
60
REGRESSION

ABBREVIATIONS USED IN TABLE

PN : Sham-operated birds.

C : Normal intact birds.

PX : Pinealectomized birds.

* : Percentage difference for pinealectomized birds, compared with normal birds.

30 : 30 days interval post-operation.

45 : 45 days interval post-operation.

60 : 60 days interval post-operation.

ablation decreased the weights of pancreas, uropygeal gland and gonads. During the recrudescent phase, pineal removal reduced the weights of the spleen and thyroid gland. However, the weight of spleen increased during both, breeding and regression periods, while that of thyroid increased during breeding, and again reduced during the regression phase. The weight of adrenal glands tended to increase after pinealectomy in the recrudescent and breeding phases while reduction in weight was the feature during the regression phase. Pancreas weight was decreased significantly during the breeding phase than at any other phase of the reproductive activity. Less reduction in uropygeal gland was observed during the breeding phase than the recrudescent and regression periods. Change in the gonadal weight was maximal during the late recrudescent and early-mid breeding phases. Pinealectomy increased the weights of spleen, adrenal and thyroid gland during the breeding period with the change in spleen weight being the most significant of the three organs. Thyroid weight shot up to a maximum (70 %) in PX60 pigeons during the breeding period. In the recrudescent period, when spleen and thyroid gained weight, the adrenal weight was reduced. Due to pineal ablation, in the regression period, spleen showed increase in its weight while adrenal and thyroid showed decreased weights.

Under natural photoperiodic conditions, the birds (pigeons) appear to have a regular cycle of body weight gain and loss. Apparently, late regression (282.72 g.) and late recrudescence / early breeding (286.00 g) appear to be the periods of maximal body weights. During the breeding and regression periods, body weights tended to remain more or less constant (248.12 $\pm$ 7.27 g).

Pinealectomy brought about fall in body weight during recrudescent and breeding periods, while in the regression period the body weight of the birds showed an increase as in the controls.

DISCUSSION

Alterations in gonadal weight and activity represent the characteristic feature of many seasonal breeders. Moreover, some of the associated endocrine and non-endocrine organs too are thought to show certain concomitant changes in response to seasonal reproductive activities of vertebrates. However, a systematic account of changes in various organs in conjuction with gonadal activity is not yet recorded fully in birds. In the course of the present study, five glandular structures have been observed alongwith the gonads during regression, recrudescent and breeding periods of the feral blue rock pigeons.

Evidently, minimal gonadal weights were observed during the regression phase and ⇘ maximal weights during the late recrudescent / early breeding periods. Two of the endocrines of profound importance in many internal homeostatic interactions - the adrenal and thyroid - have both depicted definite changes in relation to gonadal activity and functioning. Both showed a common change during the regression period marked by their maximal weights, and during the breeding, their weights decreased gradually, ultimately reaching their minimal weights in the late breeding period. A low key functioning of both the glands during the breeding season appears to be the feature and probably the gonadotrophic and/or gonadal hormones exert a negative influence on both these glands. Similar negative reciprocal relationship with the gonads has been reported for both the glands in avian species (Jallageas et al., 1978). Of the two glands, thyroid appears to be affected more profoundly as denoted by the more pronounced percentage decrease in weight (108 %) during the breeding phase, and 128 % increase in weight observed post-breeding. Definite opposite gonad-thyroid axis is further confirmed by the observed decrease in weight of the thyroid during late regression through the recrudescent and into the breeding months. However, adrenal seems to have some parallel relationship with the gonads during the recrudescent phase and may have some functional correlation with gonadal activation. Observations on

pancreas tend to indicate a tendency to maintain the same weight range all throughout the year, though the gland did depict a slight increase during the begining of all reproductive phases. Spleen and uropygeal gland have not yet been studied on a seasonal basis. Interestingly, both the organs have shown fluctuations in weight during reproductive cycle of feral pigeons. Spleen seems to be hyperactive during the recrudescent phase with about 200 % increase being registered from the low weight in the late regression period. Both breeding and regression phases were marked by progressive fall in spleen weight from the slightly higher levels during the beginning of both the phases. The increased size of spleen during the recrudescent period might implicate this organ in increased haemopoietic activities and/or immunological aspects associated with this period and is probably controlled by the gonadotrophic and adrenal hormones. In contrast, the uropygeal gland was noted to show lowest weights during early recrudescence and early breeding respectively. From thence, there was a progressive increase in weight of the gland through recrudescent and breeding periods ultimately registering the maximum during late recrudescence and early regression respectively. Though the fall in weight of this gland during the regression period and its increase during recrudescence and breeding phases definitely indicate this gland to be a probable target organ of the gonadal hormones,

the 38 % fall in weight of the gland observable during early breeding period (from the late recrudescent period) cannot as yet be understood.

Heubner (1896) described the case of a tumor of pineal gland causing precocious sexual maturation in human beings. After this startling observation, many workers attempted to study the role of pineal in various groups of vertebrate species. Shellabarger (1953), and Kitay and Altschule (1954) observed gonadal hypertrophy after pinealectomy in fowl, and gonadal atrophy after injections of pineal extract. Stalsberg (1965) observed no change in gonads after pineal ablation in fowl, while pineal stimulated gonadal maturation was reported in quail (Sayler and Wolfson, 1967, 1968). Adult rats showed increased weights of testes after pinealectomy (Motta et al., 1967). Similar effect of pineal removal was observed in mouse by Houssay et al. (1966a, 1966b). According to Goldman et al. (1979) exogenous melatonin can cause testicular regression in pinealectomized hamsters irrespective of photoperiods. Chen (1981) observed testicular regression following small doses of melatonin injections in male golden hamsters. Quite a few of these published studies reveal that the pineal gland in mammals and domestic birds has an antigonadal role. Only Homma et al. (1967) recorded inhibitory

effect of melatonin on the growth of gonads in quail. Pinealectomy was found most effective under natural photoperiods and the effect was markedly antigonadotrophic in intact weaver birds (Balasubramanium and Subramanium, 1979). According to Saxena et al. (1979) pineal was antigonadotrophic during regression phase and progonadotrophic in breeding phase in weaver birds. In the present investigation conducted under natural photoperiods in wild pigeons, pineal is found to be progonadal in nature. This is amply evident by the observed increasing gonadal regression during the recrudescent period (42 - 77 %), maintenance of maximal gonadal regression during the breeding months (75 - 66 %) and the gradually reducing incidence of gonadal regression during the regression period in PX birds as compared to the intact controls. Apparently, a progonadal role can easily be surmised. The results suggest that in wild pigeons, pineal produces some progonadotrophic factor(s) during recrudescent and breeding periods which lead(s) to gonadal activation, and the cessation of production of this pineal principle during the post-breeding period leads to gonadal regression. Motta et al. (1967) have opined that the change in gonadal weight is due to the effect of pineal on pituitary gonadotrophins. Probably these hormones are secreted more in response to pineal cues or else the effect of the trophic hormones is potentiated either directly or indirectly by increasing the sensitivity of the gonads.

Evidences from mammals suggest that the possibility of a relationship between the pineal and adrenal should not be ignored (Wurtman et al., 1968). Singh and Turner (1967) have shown that injected melatonin can cause decrease in weight of the adrenals in chicken. Cogburn and Harrison (1980) found general adrenal enlargement after pinealectomy in cockerels. Both these evidences suggest the inhibitory effect of pineal on adrenal gland in the animals. An increase in adrenal weight was the feature in PX pigeons in the current study during recrudescent and breeding periods. This increase reached its peak at the early recrudescent period. In the regression phase however, pineal ablation tended to decrease adrenal weight. These changes could be due to the absence of pineal factor(s) which probably act(s) on adrenal functioning and growth through its control on pituitary, or directly by altering the trophic hormone action which controls the adrenal functioning.

Baschieri et al. (1963) reported that daily injections of melatonin could modify the morphology of rat thyroid and its uptake of radioactive iodine. Houssay et al. (1966[a,b]) showed increase in weight of the thyroid in mice after pinealectomy. Pinealectomy was shown to stimulate an 11.8 % increase in thyroid hormone secretion rate (TSR) in rats (Ishibashi et al., 1966). The results of Narang et al.

(1967) indicated that melatonin depressed TSR, but the effect appeared to be reduced with advancing age in female rats. In domestic species of birds viz., chicken and ducks, the thyroid might act as a stimulator to the testis function, while in a number of wild species of birds (e.g. starlings, ducks) an increased thyroid activity is responsible for the seasonal regression of the testis (Jallageas and Assenmacher, 1974). Herein observed increase in thyroid weight in PX condition during both recrudescent and breeding phases (more significantly during the breeding phase) and the concomitant pronounced shrinkage of the gonads tend to strengthen the concept of an opposing thyroid -gonad axis in wild pigeons. This is further stressed by the observed pronounced decrease in thyroid weight paralleled by the narrowing of the difference in gonadal weight between PX and control birds during the regression period.

Though there have been at least some attempts to study the pineal-adrenal and pineal-thyroid axis in birds and mammals, no study is reported to-date on pineal-pancreas relationship. Present findings indicate that the pineal may have a stimulatory influence on pancreas as denoted by the reduction in pancreas weight after pinealectomy in all the three reproductive phases. Maximal reduction in pancreas weight in PX condition during the breeding period may

suggest probable parallel relationship of pancreas with gonadal activity. Two other organs (viz., spleen and uropygeal gland) which have also been studied for the sake of mere curiosity, have shown definite changes after pinealectomy. Uropygeal gland as a target organ of gonadal hormones and/or gonadotrophins is being increasingly realised (Ghosh and Bhattacharya, 1978; Kotak, 1979). Possible functioning of the gland as an accessory steroid producing organ is also being debated (Ghosh and Bhattacharya, 1978). In this respect the observed shrinkage of the uropygeal gland in PX condition during all phases of study seems to be parallel to that of the gonads. Lack of gonadotrophin hormones in PX condition might thus account for the shrinkage of this gland during recrudescent and breeding periods. However, the persistent shrinkage even during the regression phase is not compatible with this concept and may underscore the probable direct influence of some pineal principle(s) on uropygeal structure and function in wild pigeon. What has come as more surprising is the changes brought about in spleen weight of PX pigeons. We are still unaware of any humoral or other control of spleen in aves. More interesting is the differential pattern of changes recorded during the recrudescent period on one hand and breeding and regression periods on the other. Accordingly, there was a decrement in the organ weight during the recrudescent period and increment during

the other two seasons. Increase in spleen weight was very pronounced during the breeding period while it was only marginal during the regression period. These observations suggest a possible direct and/or indirect involvement of pineal in the adaptive alterations of various endocrine principles in modulating normal functional activities of the spleen. Possible involvement of gonadotrophin and gonadal hormones on spleen structure and function too cannot be overruled.

Apparent progonadal nature of the pineal in the wild pigeons and the involvement of certain pineal principles in bringing about the adaptive modulations of metabolic activity during recrudescent and breeding periods are further indicated by the observed pattern of body weight changes during the different periods. Evidently, pinealectomy brings about fall in body weights during recrudescent and breeding phases. Further, pinealectomy had no effect on body weight changes during the regression phase. This differential effect of pinealectomy tends to confirm the involvement of pineal during the active phase of breeding and its refractoriness in the regression months.