INTRODUCTION

The role of non-classical hormones emanating from thyroid and adrenal in modulating reproductive functions in mammals is gaining increasing validity. In the avian species, these glands have been reported to show seasonal variations in structure and activity in relation to breeding activities which have led to the tentative suggestions of parallel or inverse adrenal-gonad axis in some birds (Riddle et al.,1924; Legait and Legait, 1959; Fromme-Bouman, 1962; Ramachandran and Patel, 1987) and parallel or inverse thyroid-gonad axis in others (Thapliyal and Pandha, 1967 a,b; Jallageas and Assenmacher, 1973, 1974; Chandola and Thapliyal,1974; Oishi and Konishi, 1978; Jallageas et al., 1978). But these early suggestive interpretations have never been explored fully or carried forward to their logical conclusion due to inadequate experimentation or for want of systematic and detailed study in any single species. Similar is the fate of the status of pineal function in relation to reproduction inspite of the many studies which have yielded inconsistent and at times contradictory results (Ralph, 1981). Based on the disparate results obtained, pineal has been variously referred to as progonadal, antigonadal or both or even said to have no influence whatsoever. A definite role of pineal in reproduction is however highlighted by the many studies which have shown the influence of photoperiodism in regulating the annual gonadal cyclicity of temperate species of birds (Wingfield and Farner, 1980; Follett, 1980). Studies conducted in some tropical species indicate that annual gonadal cyclicity may not be directly dependent on photoperiodism; though many of them are shown to be responsive/sensitive to photoperiodic manipulations (Marshall and Disney, 1956; Lofts,1962; Epple et al.,1972; Lewis et al.,1974; Thapliyal, 1981; Chandola et al., 1985). Some sort of a link between pineal and thyroid in birds is further substantiated by the many reports suggesting alterations in thyroid activity in response to photoperiodic manipulation or the ability of functional manipulation of thyroid activity to affect normal or photoperiodically modulated gonadal cyclicity (Meier, 1969; Masuda and Oishi, 1989; Das and Chandola,1990), though the workers have not given due attention to the linkage. Moreover, the possible involvement of adrenal in this context has been more or less ignored.

It was in this behest, a concerted systematic study on a single species (male pigeon) on a seasonal basis was undertaken to gain an insight into the possible pineal-adrenal nexus in controlling gonadal functions. Though there are some reports indicating suppressive actions of pineal on thyroid and adrenal functioning in mammals and, a recent report of Chaturvedi (1984) on melatonin induced atrophy of adrenal and gonad in the common myna, there are hardly any other investigations exploring the interactions of the pineal with that of adrenal and thyroid in gonadal functioning. To this end, investigations were initiated in this laboratory on pigeons. Earlier studies dealing with surgical pinealectomy on a seasonal basis clearly established a progonadal role for the pineal, as pinealectomy (PX) in the breeding season brought about testicular regression in both wild and domestic pigeons (Patel *et al.*,1988; Ramachandran and Patel, 1987; Ramachandran *et al.*,1987). On a seasonal basis, intact feral pigeons show decreased thyroid activity and increased adrenal activity in the breeding season and vice-versa in the non-breeding season. These normal seasonal changes were reversed in PX birds, with the result thyroid activation and adrenal inhibition occurred in the breeding season and vice-versa in the non-breeding season (Patel *et al.*,1985). A later study showed that adreno-cortical suppression in the breeding season also led to gonadal regression (Ayyar, 1987; Ayyar *et al.*, 1992). Overall, these findings indicate a parallel pineal-adrenal-gonad axis and an inverse pineal-thyroid axis. These studies on feral pigeons clearly showed a modulatory influence of pineal on the adrenal and thyroid functions besides gonadal functions. Possibility of pineal-thyroid and pineal-adrenal interactions has been suspected to occur even in mammals, though the interactions are not very clearly specified (see Johnson, 1981). The involvement of adrenal and thyroid in the reproductive activity of birds has been reported (Pathak and Chandola, 1983; see Chaturvedi, 1993; Thapliyal, 1993).

To clearly ellucidate the relationship between pineal, adrenal and thyroid, it was decided to test the influence of functional manipulation of adreno-cortical activity on the reproductive status of both intact and PX male pigeons on a seasonal basis. Since a parallel adrenal-testes relationship is already established in this species, it was thought meaningful to study the influence of adrenocortical suppression in the breeding season and that of exogenous CORT administration during the regression and quiescent phases. Simultaneously, the influence of these experimental manipulations on thyroid activity as revealed by its histoarchitecture as well as serum T_4 level has also been evaluated to decipher the possible relationship between adrenal and thyroid. It is envisaged that these experimental evaluations would provide an overall understanding of the pineal, adrenal and thyroid interactions in regulating seasonal testicular activity in the male feral pigeons and as such forms the subject matter of chapters I and II.

Seasonal reproductive cyclicity in birds as in mammals has been reported to be regulated by many factors. What has been interesting in this context is an understanding of the regulatory mechanisms involved in seasonal gonadal recrudescence, regression and maintenance of the quiescent state. Number of mechanisms have been suggested to explain the inactiveness of the gonads in the non-breeding phase like, environmental factors, lack of gonadotrophin releasing factors, non-secretion of pituitary gonadotrophins and insensitiveness or non-responsiveness of the gonads to respond to the gonadotrophic hormones as well as altered feedback sensitivity of the hypothalamus and Pituitary (Benoit et al.,1950;Lofts and Marshall,1958; King et al.,1966; Murton and Westwood, 1977; Follett and Robinson, 1980).

In general, though the absence of gonadotrophic hormones in the non-breeding season is an acceptable fact, the altered sensitivity of the gonads to gonadotrophic hormones is a concept that needs proper scrutiny and could be a factor of significance only in a particular species. Hence, it was thought desireable to test the influence of small doses of gonadotrophins given in the non-breeding phase on the functional status of the testes. Though, the literature on this aspect is replete with reports on the influence of higher doses of gonadotrophins in different birds, a low dosage regime has been chosen specifically to test the sensitivity of the testes in the quiescent phase, as higher doses are likely to exert some influence even in an insensitive gonad. . Concurrently, apart from the functional status of the testes, the activity of adrenal and thyroid has also been investigated so as to unravel the existence of intricate relationship between gonadotrophins and adrenal and thyroid in relation to breeding. Since adrenal activity has been known to parallel testicular activity, simultaneous administration of CORT and gonadotrophic hormones has also been attempted to elucidate the possible permissive infulence of CORT on the action of gonadotrophin on testes (chapter V). Earlier studies from this laboratory in intact and PX birds on seasonal basis have revealed significant alterations in carbohydrate metabolism involving tissue glycogen content and plasma glucose level (Patel et al.,1983; 1988; Patel and Ramachandran, 1987; Ramachandran and Patel, 1987). Besides, adrenocortical suppression in the breeding season has also been reported to affect carbohydrate metabolism (Ayyar, 1987; Patel, 1993).Carbohydrate metabolism seems to have a definite relationship in this species. To have a better understanding of this aspect, circannual variations in tissue glycogen content, blood glucose and hepatic phosphorylase and G-6-P'ase activity need, to be studied. This could give a clue to the extent of involvement of carbohydrate reserves on seasonal reproductive activities. Moreover, pineal-adrenal interactions on carbohydrate metabolism also need to be studied. To this end, it was decided to evaluate the impact of functional manipulation of adrenocortical activity in the form of adrenocortical suppression in the breeding season or exogenous CORT administration in the regression and quiescent phases, on carbohydrate metabolism in intact and PX pigeons. The findings of these investigations are reported in Chapter III.

Since it is known that carbohydrate metabolism shows seasonal variations in terms of breeding activities, as well as in birds which are pinealectomised or rendered hypocorticalic, the possible influence of administration of gonadotrophic hormones in the non-breeding season neeeded to be assessed, to see whether the gonadotrophin induced activation of the gonads is parallelled by adaptive changes in carbohydrate metabolism and as such has been carried out (Chapter VI).

Like the tissue stores of carbohydrates, ascorbic acid (AA) content of liver, muscle, adrenal and testis has also been reported to show variations with respect to the functional status of the gonads. Both PX, melatonin administration, induced hypocorticalism as well as hypercorticalism have all been also known to induce changes in tissue AA content (Stubbs and McKerynan, 1967; Dieter, 1969; Dieter and Breitenbach, 1971; Patel, 1982, Ayyar, 1987; Joseph and Ramachandran, 1991; unpublished observation).

The changes in tissue AA content is generally correlatable with the metabolic status as well as steroidogenic status. Hence, the changes in tissue AA content (liver, adrenal and testis)have been assessed in both intact and PX pigeons subjected to functional manipulation of adrenocortical activity on a seasonal basis as well as in pigeons treated with exogenous gonadotrophins in the quiescent phase. Moreover, the circannual variation in tissue AA content is attempted to gauge a clear seasonal prespective. The findings are reported in chapters(IV and VII).

In a nutshell, the thrust of the experimental investigations of the present thesis is -

1. To assess the relative importance of adrenal corticoids in regulating seasonal testicular activity and its relationship with pineal.

2. To understand the impact of altered functional status of adrenal on thyroid and its relationship with testicular functions and also the possible interaction with pineal.

3. To evaluate the circannual variation in carbohydrate metabolism and tissue AA content, as well as to assess

the impact of functional manipulation of adrenocortical activity on the same in relation to pineal.

4. The influence of gonadotrophic hormones on testicular recrudescence in the quiescent phase as well as to understand the interrelationship between gonadotropins and adrenal and thyroid activity.

5. To see whether administration of gonadotropins in the non-breeding season can alter carbohydrate metabolism and tissue AA contents.

Chapter I

MANIPULATION OF ADRENOCORTICAL FUNCTIONS: ALTERATIONS IN TESTES, ADRENALS AND THYROID OF INTACT AND PINEALECTOMISED PIGEONS IN THE BREEDING AND REGRESSION PHASES.

The adrenal-gonad axis has been studied in various vertebrate groups and, many of the reports suggest adrenal activity to be parallel to gonadal cyclicity in fishes, reptiles, birds and mammals (Gabe, 1970; Moens and Coessens, 1970; Nalini and Dixit, 1976; Datta, _et al_., 1978; Munshi _et al_.,1978; Pankakoshi and Klans, 1982; Patel, _et al_., 1985; Ramachandran and Patel, 1986; Chaturvedi, 1993). However, an inverse relationship between adrenal and gonad has also been reported (Soule and Assenmacher, 1966; Assenmacher and Boissin, 1972; Bengt, 1979; Chaturvedi, 1993). Similarly the relationship between thyroid and gonad in birds is also found to be differential with a parallel relationship in some (Chaturvedi and Thapliyal, 1980; Thapliyal _et al_., 1982;Ramachandran and Patel, 1986) and an inverse relationship in others (Thapliyal and Pandha, 1967; Chandola and Thapliyal, 1974; Jallageas and Assenmacher, 1974; Jallageas _et al_., 1978; Oishi and Konishi,1978). More confusing is the picture regarding the pineal-gonad axis, as varying effects ranging from progonadal to antigonadal or progonadal in one season and antigonadal in other or even no relationship at all have been reported (Stalsberg, 1965; Sayler and Wolfson, 1967, 1968; Pitis, 1970; Joss,1973; Saxena <u>et</u> <u>al</u>.,1979; Johnston and Zucker, 1980).

Studies conducted in this laboratory in the past, intended to understand the involvement of these three endocrines in the seasonal testicular cyclicity of the blue rock pigeon, <u>Columba</u> <u>livia</u>,have revealed parallel pineal-adrenal-testes and inverse pineal-thyroid-testes relationships (Patel <u>et</u> <u>al</u>.,1985; Ramachandran and Patel, 1986). Further, pinealectomy (PX) or adrenocortical suppression by dexamethasone (DXM) has been shown to induce testicular involution in the breeding season. A recent study has also shown PX induced testicular regression to be accompanied by increased thyroid activity and reduced adreno-cortical activity (Patel,1993;Patel <u>et</u> <u>al</u>.,1993). In the light of these observations suggesting a definite relationship between pineal, thyroid and adrenal in the reproductive functions of male pigeons, an understanding of the exact interaction between these three non-classical endocrine glands in the various phases of testicular cyclicity needed to be deciphered. The present investigation was designed in this context, which involved studying the histomorphological changes and serum hormone profiles of Thyroxine (T_4) and corticosterone (CORT) under conditions of adrenocortical suppression by DXM in the breeding season and CORT treatment in the regression phase. Further, the influence of CORT administration to PX pigeons in both seasons has also been assessed.

MATERIAL AND METHODS:

Procurement and maintenance of pigeons.

Adult feral blue rock pigeons, Columba livia, in the weight range of 250-300g were procured from a local animal dealer for the present study. The birds were housed in a well ventillated aviary with food and water *ad libitum*. The birds were sexed by endoscopy and only males with similar testicular size were used for the experiments after a week of acclimation.

Experimental Setups :

In the breeding phase (March), a total of 36 male pigeons were divided into six groups of 6 each. Two female birds were kept per group to maintain the normal testicular activity.

Group I (Control : C). These birds were given daily injections of 0.1ml of saline (0.9%) at 17.00h.

Group II (Dexamethasone : DXM). These birds were given injections of 40µg DXM in 0.1ml at 17.00h and served as the adrenocortical suppression group.

Group III (Pinealectomy/ised: PX). These birds were subjected to surgical pinealectomy as per the method developed in this laboratory(Patel *et al.*, 1983) and served as pinealectomised group.

Group IV (Sham pinealectomy : SPX). These birds were sham operated and served as sham controls.

Group V (PXV). Pinealectomised birds were given daily injections of propylene glycol in saline (vehicle) and served as control for group VI.

Group VI (PX+CORT): Pinealectomised birds were given daily injections of 2µg (CORT) in 0.1ml vehicle at 09.00h.

All treatments were given intraperitoneally (ip) for 30 days. However, none of the parameters studied presently showed any alteration between Group I and Group IV and Groups III and V, and hence only data of Group I and Group III are presented.

In the regression phase (June), a total of 42 male pigeons were divided into seven groups of 6 each. Two female birds were kept per group.

Group I (Control : C). These birds were given daily injections of 0.1ml saline.

Group II (CV). Intact birds given daily injections of the vehicle at 09.00h.

Group III (CORT). These birds were given daily injections of 2μg CORT in 0.1ml vehicle at 09.00h.

Group IV (PX). These birds were subjected to surgical pinealectomy.

Group V (SPX). These birds were sham operated and served as sham controls.

Group VI (PX+CORT): Pinealectomised birds given daily injections of 2μg CORT in 0.1ml vehicle at 09.00h.

Group VII (PXV): Pinealectomised birds given daily injections of the vehicle.

All treatments were given intraperitoneally (ip) for 30 days. However, none of the parameters studied presently showed any alteration between Group I and II and Group V and VII and hence only data of Group I and Group V are presented.

[illegible]

Preparation of Solutions :

DEXONA (Dexamethasone disodium phosphate; Cadila laboratories, Ahmedabad, India) was procured from the local market. The required concentration was prepared by diluting it with 0.9% Saline.

What [illegible]

Corticosterone (CORT) :

Corticosterone procured from Sigma chemicals St. Louis, USA was used for the experiment. The required quantity of the same was dissolved in 0.5ml propylene glycol and then made upto the required concentration with 0.9% Saline.

PARAMETERS AND METHODOLOGY OF EVALUATION :

Gravimetry : Immediately after decapitation under mild anaesthasia, the viscera was cut open and testes, adrenals and thyroid were quickly excised, blotted free of blood and tissue fluid and weighed on a digital mettler balance. The relative weights are expressed as percentage of body weight.

Histology: Tissues to be processed for histological studies were fixed in Bouins fixative and processed routinely. Paraffin sections of 5μ thickness were out on a microtome and stained with haematoxyline-eosin and mounted in DPX.

Serum hormone levels : Circulating levels of T_4 was analysed using RIA kit provided by radiopharmaceutical division, BARC, Bombay. The level of T_4 was expressed as ng/ml. Corticosterone was determined by the flourimetric method of Mattingly (1962), and expressed as μg/dl.

Statistical analysis : In the present study, statistical significance for all the quantitative data was determined using students *t*-test at $P < 0.05$. The data was also subjected to ANOVA.

RESULTS :

Gravimetric Changes : Hypocorticalism induced by DXM in the breeding season decreased the weight of testes by more than 40%. However, hypercorticalism induced by CORT during the regression phase did not alter the weight of testes. Pinealectomised birds given CORT in both the seasons, could not prevent the loss of weight occurring due to Px. Treatment with DXM in the breeding season and CORT in the

Relative weight

(mg/100g body wt.)

Treatments	Testes	Adrenals	Thyroid
Control	127.58 ± 1.42	9.80 ± 0.42	5.17 ± 0.32
DXM	70.74* ± 1.74	6.10* ± 0.41	5.94* ± 0.29
PX	24.41 ± 1.18	6.51 ± 0.34	7.38 ± 0.28
PX+CORT	36.23* ± 1.93	8.15* ± 0.34	6.54 ± 0.62

Table 1a: Changes in relative weight of testes, adrenals and thyroid of normal and PX pigeoins subjected to functional manipulation of adrenals in the breeding phase.

(* Significant at $P < 0.05$; values are $\bar{x} \pm$ SEM)

Relative weight

(mg/100g body wt.)

Treatments	Testes	Adrenals	Thyroid
Control	25.16 ± 1.70	7.63 ± 0.19	7.24 ± 0.20
CORT	24.74 ± 1.17	5.40* ± 0.15	6.00* ± 0.17
PX	14.63 ± 1.33	5.97 ± 0.28	7.54 ± 0.18
PX+CORT	19.98* ± 1.56	6.12 ± 0.29	7.08* ± 0.28

Table 1b: Changes in relative weight of testes, adrenals and thyroid of normal and PX pigeons subjected to functional manipulation of adrenals in the regression phase.
(* Significant at $P < 0.05$; values are $\bar{x} \pm$ SEM)

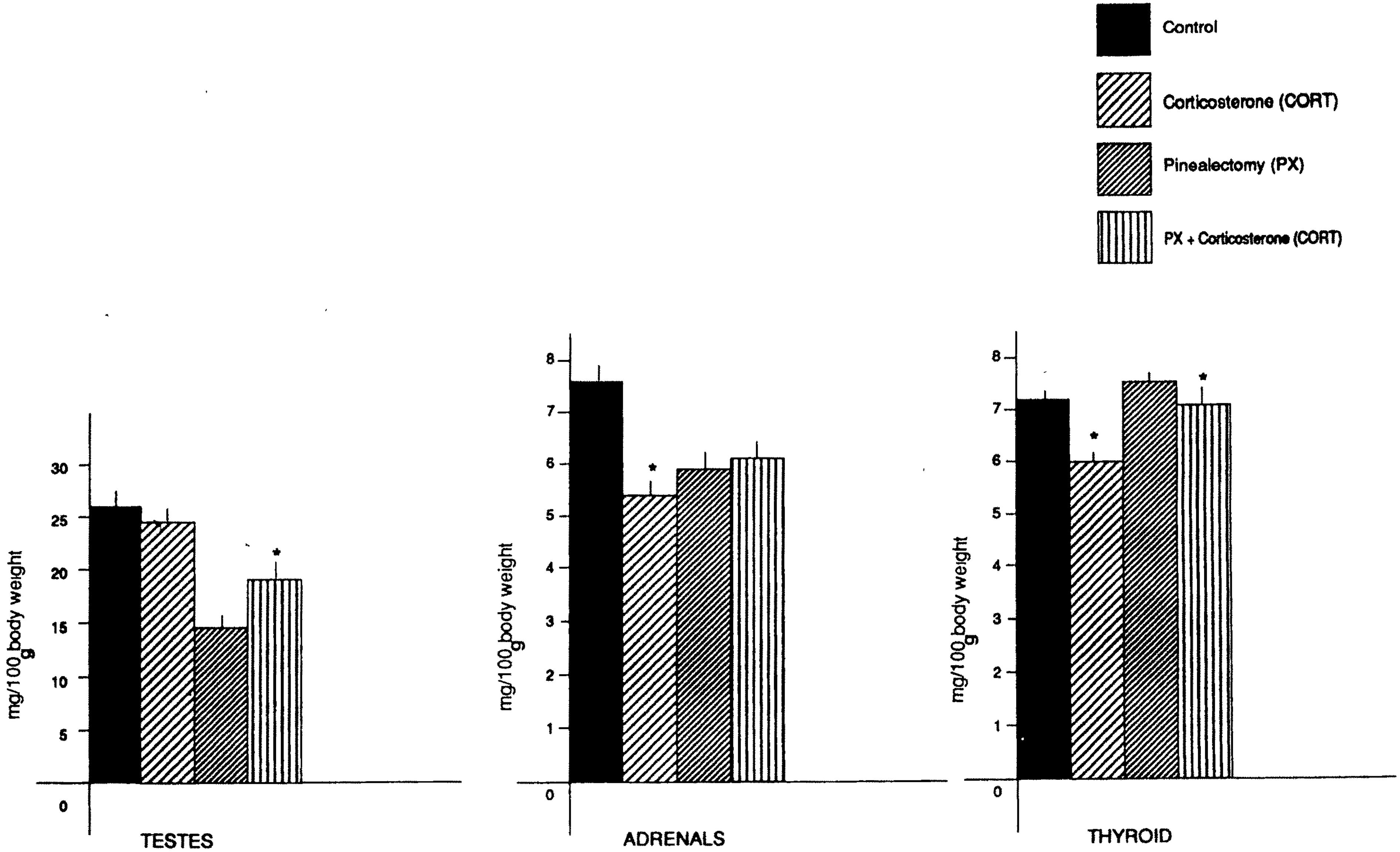


Fig. : 1 B Changes in relative weights of testes, adrenals and thyroid of intact and PX pigeons treated with corticosterone (CORT) in the regression phase (* = Significant at $P < 0.05$; values are $\bar{x} \pm$ SEM)

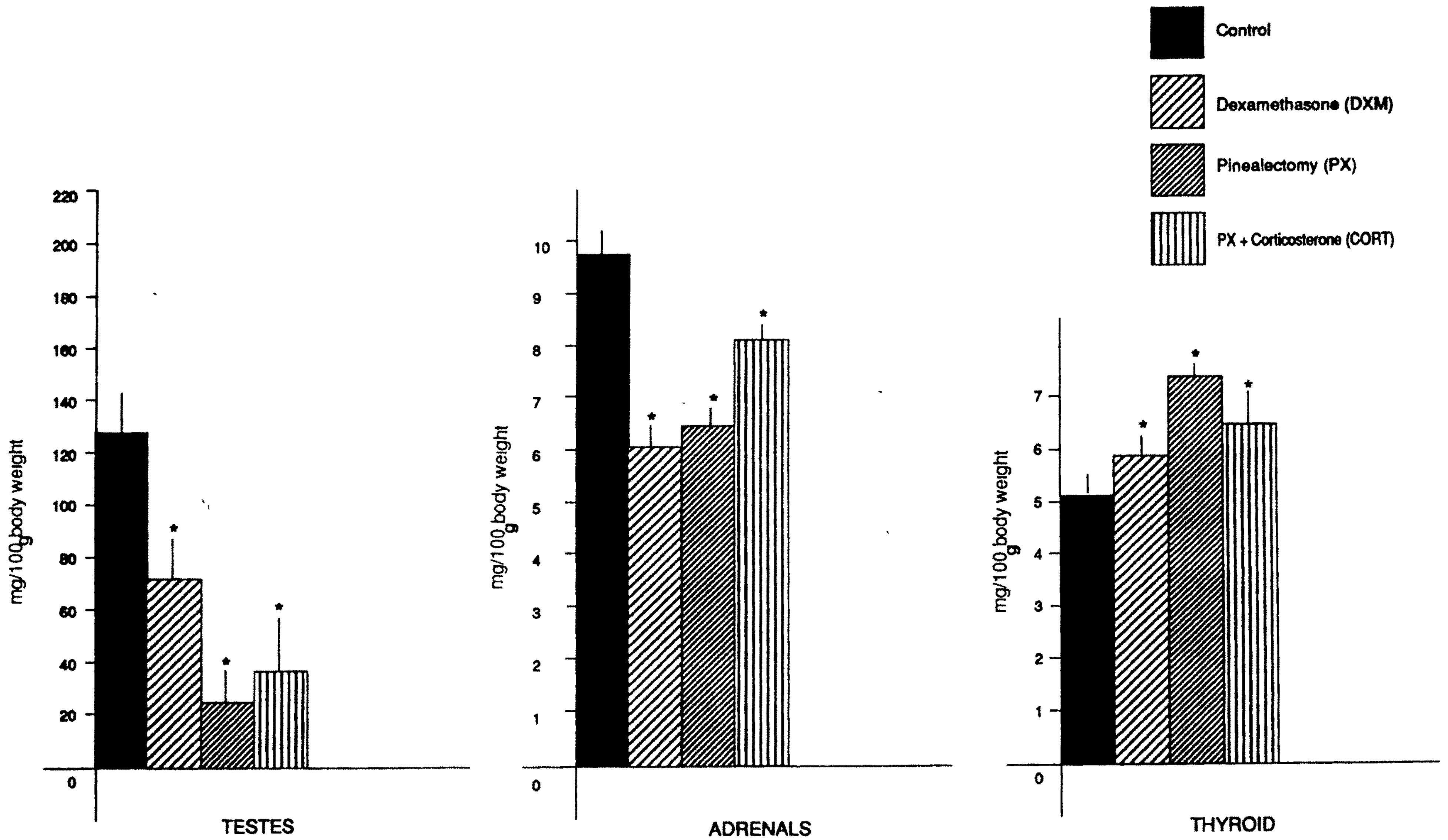


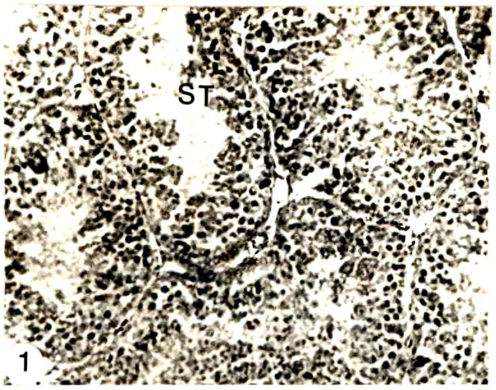
Fig. : 1 A Changes in relative weight of testes, adrenals and thyroid of intact and PX pigeons subjected to functional manipulation of adrenal in the breeding phase (* = Significant at $P < 0.05$, values are $\bar{X} \pm$ SEM) regression phase significantly decreased the weight of adrenals and thyroid. Pinealectomised birds treated with CORT in the breeding season showed significantly decreased adrenal weight, while there was no significant change in the regression phase. Thyroid weight was increased in DXM treated intact birds in the breeding season and decreased with CORT treatment in the regression phase. However, PX birds treated with CORT did not show any weight alteration in either season.(Table 1a; 1b; Fig. 1A,1B).

Histological changes :

Testis : The testis of control birds during the breeding season showed enlarged fully active seminiferous tubules. The tubules depicted all stages of spermatogenesis including spermatids and sperm bundles. The small interstitial cells were compressed in between the enlarged tubules. Testis of DXM treated birds during the breeding season showed varying degrees of degeneration. Hypertrophied spermatogonial cells with pyknotic nuclei were seen. Inactive interstitial cells appearing like fibroblasts were also noticeable. Testis of control birds during the regression phase had greatly shrunken seminiferous tubules with large intertubular spaces. The tubules were lined with only a basal layer of gonial cells and the interstitial cells were also regressed. Corticosterone treatment did not change the condition of the

PLATE I

Figures 1-6	Photomicrographs of sections of testes of control and experimental birds (200 X).
Fig.1	Testis section of control birds during the breeding season showing seminiferous tubule (ST) with active spermatogenesis.
Fig.2	Testis section of Dexamethasone (DXM) treated birds during the breeding season showing regressed tubules with degenerating germ cells with pyknotic nuclei.
Fig.3	Testis section of Pinealectomised (PX) birds during the breeding season showing regressed tubules with disrupted spermatogenesis.
Fig.4	Testis section of control birds in the regression phase showing highly regressed seminiferous tubule with large intertubular spaces.
Figs 5 & 6	Testes section of control and PX pigeons respectively treated with corticosterone (CORT) in the regression phase. Note the unchanged condition in the former and the greater regressive changes in the latter.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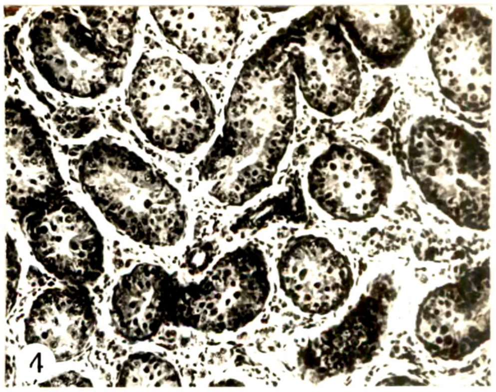
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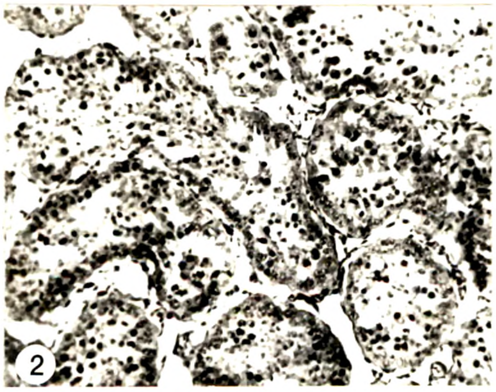
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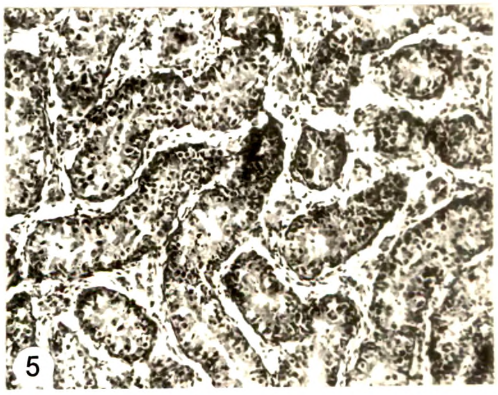
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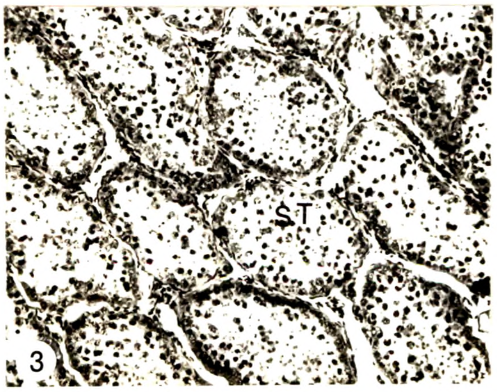
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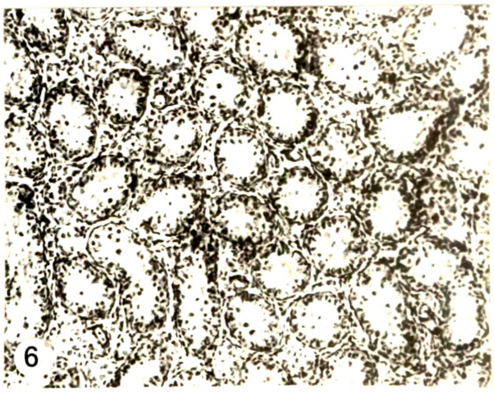
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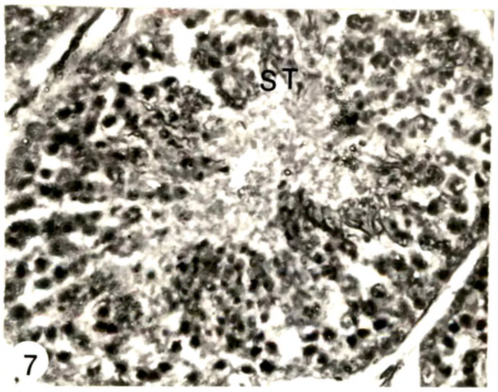
PLATE II

Figures 7-12	Photomicrographs of sections testis of control and experimental birds (400 X)
Fig.7	Testis section of control birds in the breeding phase. A single seminiferous tubule (ST) showing active spermatogenesis.
Fig.8	Testis section of Dexamethasone (DXM) treated birds in the breeding phase showing regressed ST with degenerating germ cells.
Fig.9	Testis section of pinealectomised (PX) birds in the breeding phase, showing regressed ST and degenerating germ cells.
Fig.10	Testis section of control birds in the regression phase showing regressed tubules with degenerating germ cells. Note the hypertrophied interstitial cells (IC) in some parts.
Figs. 11 & 12	Testis section of control and PX birds respectively in the regression phase, treated with corticosterone. Note the regressed condition of the tubules in both.



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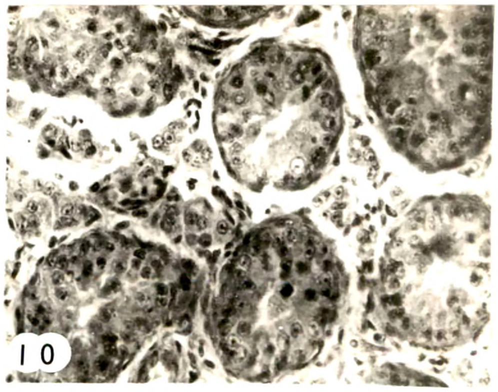
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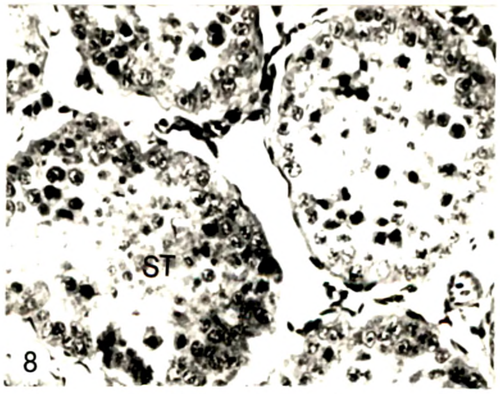

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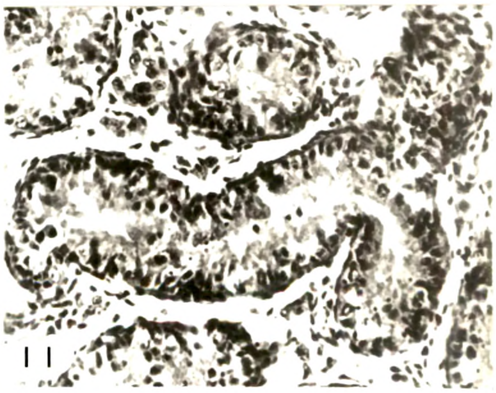
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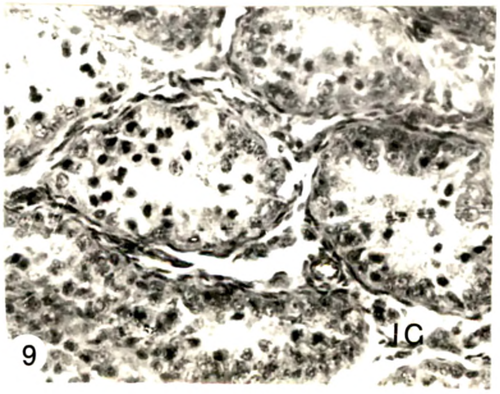

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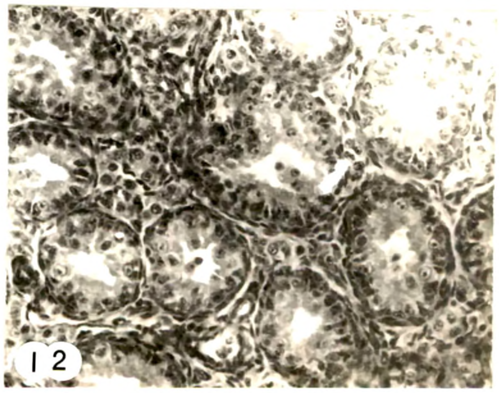
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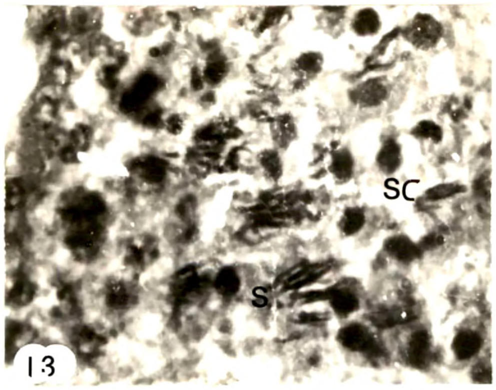
PLATE III

Figures 13 - 18	Photomicrographs of section of testis of control and experimental birds. (640 X)
Fig.13	Part of a seminiferous tubule of a control birds in the breeding phase showing spermatids (S) and spermatocytes (SC).
Fig.14	A single tubule of DXM treated bird showing pyknotic nuclei (PN).
Fig.15	A single ST of PX birds showing degenerating germ cells (DG) and pyknotic nuclei (PN).
Fig.16	A single tubule of a control bird in the regression phase showing degenerating germ cells and some hypertrophied interstitial cells (IC).
Figs 17 & 18	Enlarged version of the regressed tubule of control and PX pigeons respectively treated with CORT in the regression phase. Note the degenerating germ cells (DG).



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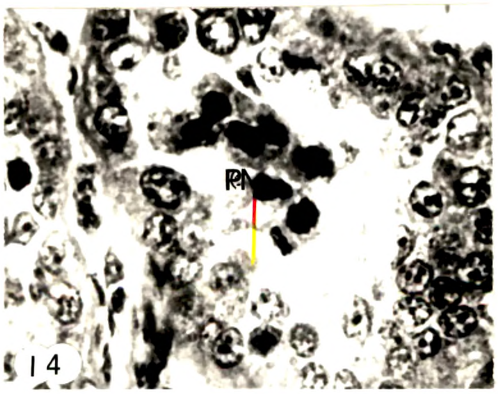

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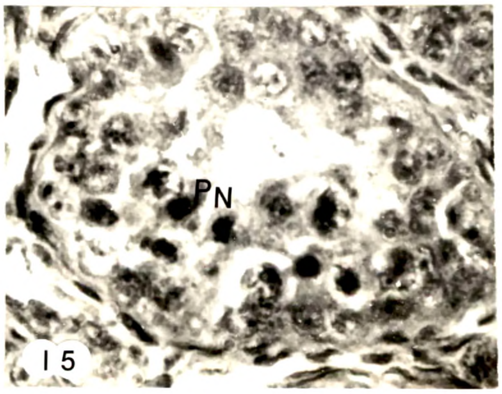

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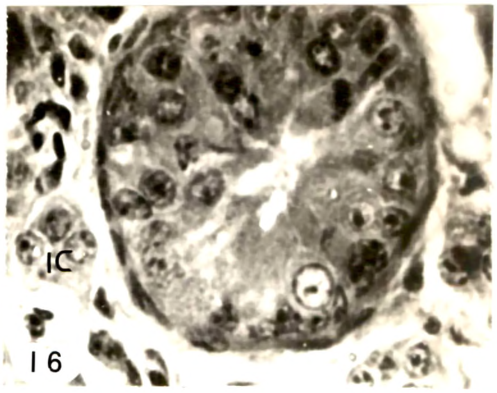

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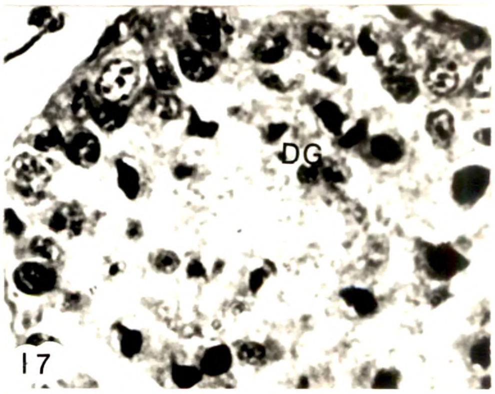

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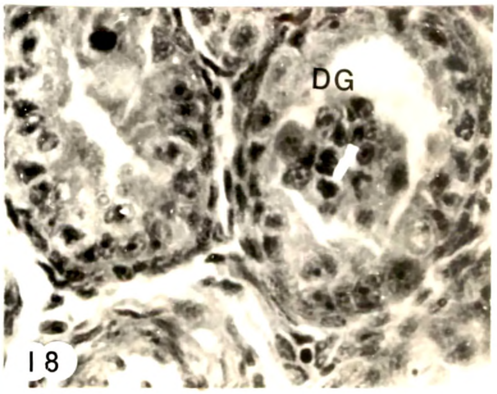

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regressed testis. Pinealectomy in the breeding season showed regressive changes as marked by shrunken tubules with one or two layers of hypertrophied germ cells. Interstitial cells were also hypertrophied. However, pinealectomy in the regression phase showed extensive involutionary changes. The tubules showed clumps of degenerating germ cells in the lumen and the interstitial cells were hypertrophied. Corticosterone treatment to PX birds during both the seasons did not have marked effect on the condition of the regressed testis. (Plates I to III)

Adrenal : The adrenal of control birds in the breeding season showed large active cortical cords with a higher cortico-medullary ratio. Dexamethasone treatment brought about regression of the cortical cords. The adrenal of control birds during the regression phase showed slightly shrunken cortical cords with reduced cortico-medullary ratio. However, CORT treated birds showed active cortical cells with enlarged cortex. Pinealectomy in both the seasons brought about cortical regression while, CORT treatment to PX birds in both the seasons was marked by regressed inactive cortex with equal cortico -medullary ratio. (Plate IV)

Thyroid : The thyroid of control birds in the breeding season was marked by large turgid follicles, full of colloid and the follicular epithelium was flat. Thyroid of DXM

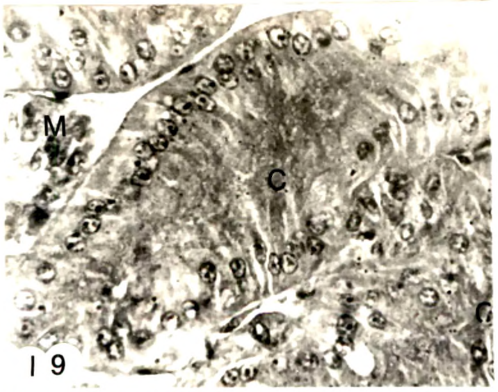
PLATE IV

Figures 19-26	Photomicrographs of section of adrenal of control and experimental birds (200 X).
Fig.19	Section of adrenal of control birds in the breeding phase, showing prominent cortical cords (C). Note the active state of the cortical cells.
Fig.20	Section of adrenal of DXM treated birds in the breeding phase. Note the greatly regressed state of the cortical cords and inactive state of the cortical cells.
Fig.21	Section of adrenal of PX pigeons in the breeding phase showing regressive changes in cortical cords.
Fig.22	Adrenal of PX pigeons treated with CORT in the breeding season. Note the better state of the cortical cells.
Fig.23	Adrenal of control pigeon in the regression phase. Note the regressed cortical cords and inactive cells.
Fig.24	Adrenal of control birds treated with CORT in the

regression phase. Note the more active state of the cortical cords.

Fig.25 Adrenal of PX pigeons in the regression phase. Note the regressed cortical cords.

Fig.26 Adrenal of PX pigeons treated with CORT in the regression phase. Note the more or less unchanged situation.



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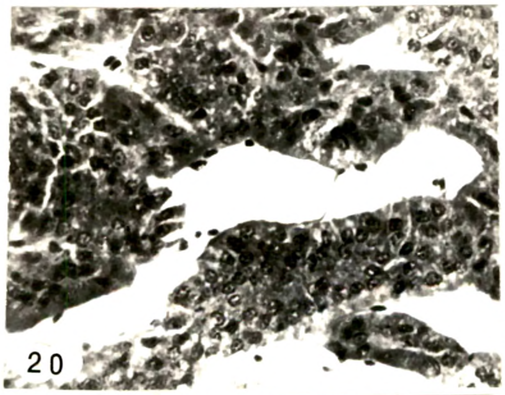

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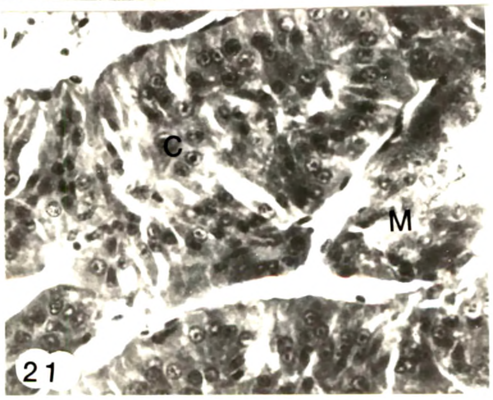

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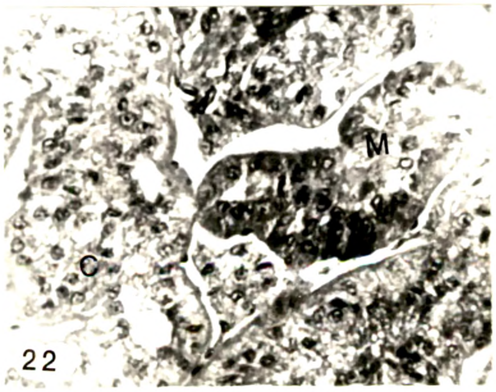

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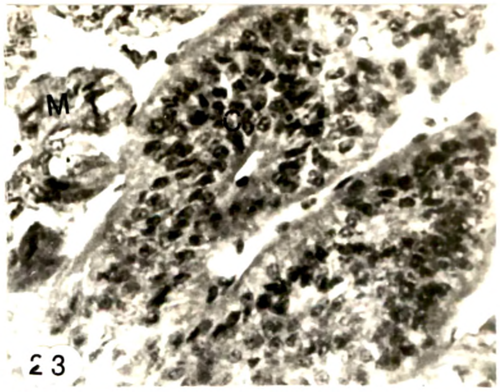

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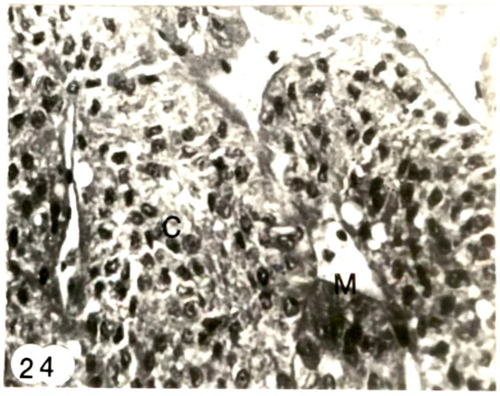

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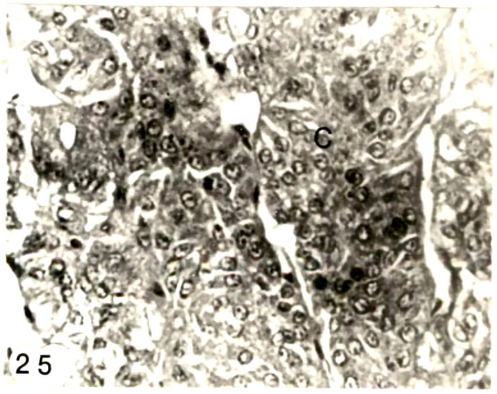

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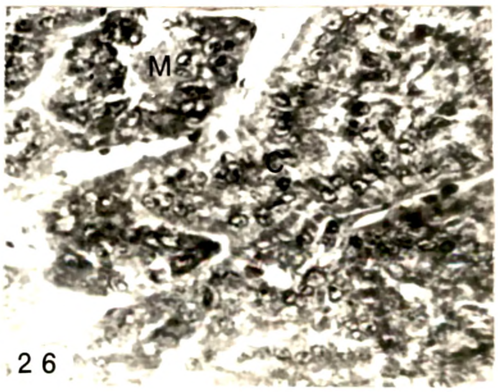

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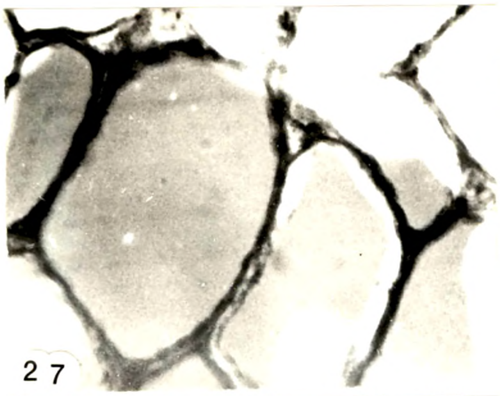
PLATE V

Figures 27-34	Photomicrographs of sections of thyroid of control and experimental birds (260 X)
Fig.27	Thyroid of control birds in the breeding phase showing well formed follicles, filled with colloid and thin epithelium.
Fig.28	Thyroid of DXM treated birds in the breeding phase. Note colloidal depletion from the follicles and increased cell height.
Fig.29	Thyroid of PX pigeons in the breeding season showing colloid depleted follicles and increased cell height.
Fig.30	Thyroid of PX pigeon treated with CORT in the breeding season. Note the unchanged state.
Fig.31	Thyroid of control birds in the regression phase showing colloid depletion and increased cell height.
Fig.32	Thyroid of control bird treated with CORT in the regressing phase. Note the reduced cell height and

retention of colloid in the follicles.

Fig.33 Thyroid of PX birds in the regression phase showing colloid depleted follicles.

Fig.34 Thyroid of PX birds treated with CORT in the regression phase.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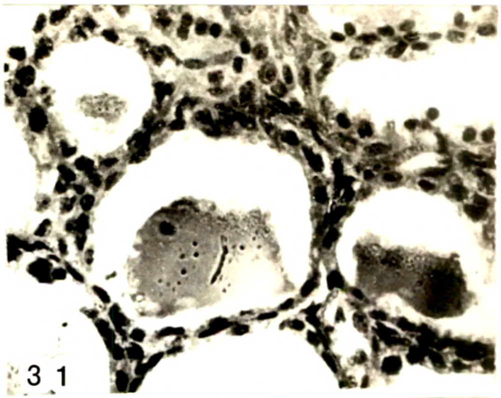
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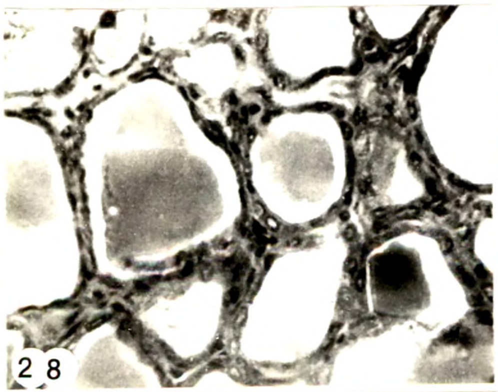
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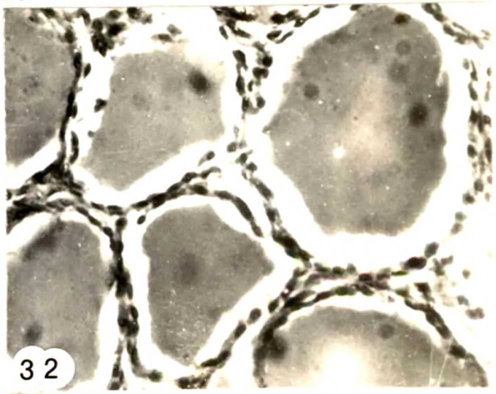
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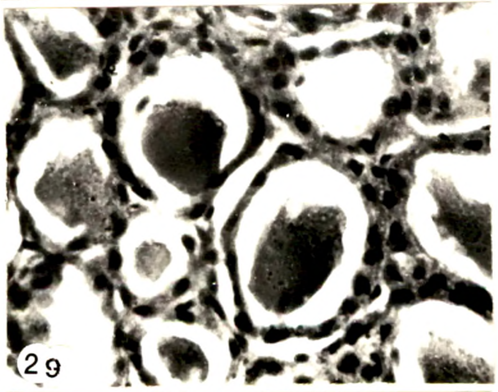
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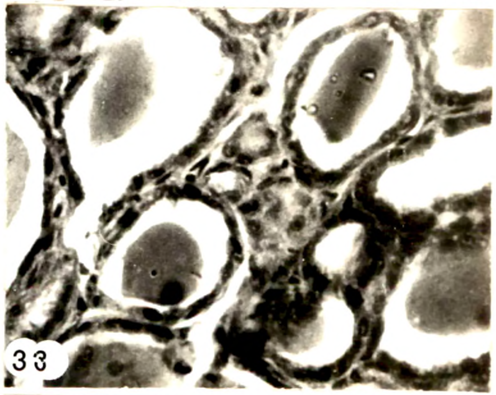
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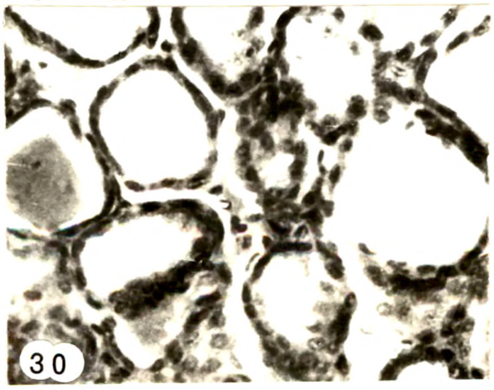
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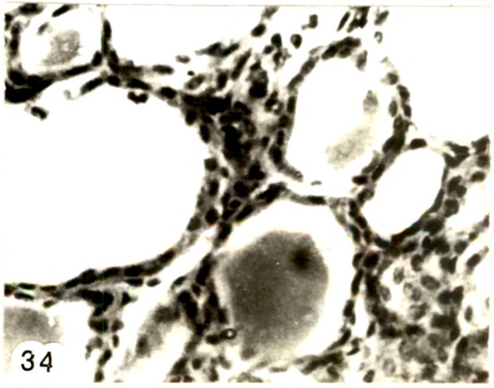
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treated birds showed a mixed population of empty and colloid filled follicles with flat follicular epithelium. Thyroid of control birds in the regression phase showed depletion of colloid from the follicles with cuboidal follicular epithelium. Birds treated with CORT had colloid filled follicles. The follicular epithelium of PX birds during breeding as well as regression phases was cuboidal and the follicles showed depletion of colloid. Corticosterone treated PX birds in both the seasons also showed increased cell height of the follicular epithelium. Most of the follicles were devoid of colloid and the lumen of many follicles was obliterated. (Plate V)

Serum hormone levels :

The serum level of T_4 and CORT in control birds during breeding season was 16.46 ± 2.14 ng/ml and 8.95 ± 1.48 µg/dl and, the same during the regression phase was 23.22 ± 2.09ng/ml and 6.27 ± 1.23 µg/dl respectively. Birds treated with DXM in the breeding season had higher T_4 level and lower CORT level. Pinealectomy in either season increased T_4 level and decreased CORT level. Pinealectomised birds administered with CORT in either season showed no significant change in the serum hormone levels of T_4 and CORT. However, CORT administration to intact birds during the regression phase decreased serum T_4 level and increased CORT level

BREEDING			REGRESSION		
Treatments	T_4 (ng/ml)	CORT µg/dl	Treatments	T_4 ng/ml	CORT µg/dl
C	16.46	8.95	C	23.22	6.27
	± 2.14	± 1.48		± 2.09	± 0.47
DXM	19.98*	5.80*	CORT	17.88*	7.29*
	± 3.80	± 1.29		±1.81	± 0.80
PX	22.84	6.72	PX	23.98	5.01
	± 2.81	± 1.34		± 2.17	± 0.73
PX+CORT	21.94	7.32*	PX+CORT	22.77*	5.98*
	± 1.34	± 1.46		± 1.18	± 0.63

Table Ic : Alterations in serum levels of T_4 and CORT of PX and intact pigeons subjected to functional manipulations of adrenal in the breeding and regression phases. (* = Significant at $P<0.05$; values are $\bar{X} \pm$ SD) marginally. (Table 1c).

DISCUSSION :

Previously it was shown that adrenocortical suppression in the pigeon leads to regression of the testes in the recrudescent and breeding phases (Ayyar, 1987; Ayyar _et al._,1992). It was also shown that PX induces involution of the testes accompanied by adrenocortical regression (Patel _et_ _al._,1985; Ramachandran and Patel, 1986). The above observation tended to suggest the PX induced testicular involution to be related to the reduced adrenocortical activity. Confirmation of this concept as well as the involvement of melatonin and CORT individually or even their interactions needed experimental scrutiny. The present experimental manipulations intended to unravel the interactions or the interrelationships between pineal and adrenal have revealed intricate and interesting relationships not only between these two glands but also with thyroid in regulating testicular functions in the pigeon. The reduced serum CORT level indicates a parallel adrenal-testis relationship and lends credence to the earlier concept of PX induced regression of the testis to be due to reduced adreno-cortical activity. However, CORT administered to PX birds did not prevent the involutionary changes in the testis, thereby negating the above concept.

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1993; present results). However, the ability of CORT to reduce serum T_4 level in intact birds and, its inability to do so in PX birds as observed in the present study, suggest no direct action of CORT on the HHT axis. It is clearly inferable that the action of CORT is to potentiate the inhibitory action of melatonin on the HHT axis. It is also presumable that melatonin has a stimulatory influence on the Hypothalamo-Hypophysial-Adrenal (HHA) axis. This is confirmed by the observed decrease in serum CORT level subsequent to pinealectomy. The higher CORT level in the recrudesecent and breeding seasons acting synergistically with melatonin potentiates the inhibitory influence on the HHT axis resulting in reduced serum T_4 level which keeps the gonads functioning. An interesting observation is the prominent hypertrophy of the follicular epithelium of thyroid gland in PX pigeons treated with CORT in the regression phase. Such a change was not evident in PX birds or, in intact pigeons treated with CORT in the same phase. This would suggest increased TSH stimulation in PX birds treated with CORT. Evidently, CORT seems to decrease the sensitivity of hypothalamo-hypophysial axis to the negative feedback action of thyroxine resulting in elevated TSH secretion and hypertrophy of the follicular epithelium.Though exogenous CORT administration in the regression phase could suppress the HHT axis and reduce serum T_4 level, there was no apparent effect on the

Any other pineal factor?

Pertinently, both PX, as well as DXM induced hypocorticalism, depleted the colloidal content from the thyroid follicles which was reflected in the elevated serum T_4 levels in both these groups of birds. Ongoing studies in this laboratory have shown regressior of testes in the breeding season in pigeons administered with T_4. Viewed in this perspective, the testicular regression brought about by either PX or induced hypocorticalism seems to be essentially due to the elevated serum T_4 level. Substantiation to this concept of T_4 induced testicular regression is provided by the reports of gonadal involution due to exogenous T_4 administration and prevention of gonadal regression by prior thyroidectomy in some birds (Chandola and Thapliyal, 1978; Chandola and Bhatt, 1982; Chandola et al., 1982; see Pathak and Chandola, 1983). The Hypothalamo-Hypophysial-Gonad (HHG) axis seems to be sensitive to the inhibitory influence of increased levels of T_4 in the breeding season. In fact, such an elevation in serum T_4 level was purported to be the factor mediating gonadal regression at the end of the breeding season in the pigeon (Patel, 1993).

From the results obtained in the present study it appears that both melatonin and CORT may have a direct suppressive effect on the HHT axis. This is confirmed by the ability of both melatonin and CORT to bring about colloidal retention in the thyroid follicles and reduce serum T_4 level (Patel, regressing testes. It is likely that once the HHG axis has been suppressed by T_4 action at the onset of regression, lowering of T_4 level thereafter is without any effect as the HHG axis is rendered insensitive. Lack of studies of this nature in any vertebrate group including birds precludes any meaningful comparative discussion of the data.

Overall, the present study suggests an important role for pineal gland and its hormone melatonin in regulating the reproductive activity of feral blue rock pigeon by modulating the functions of the HHA, HHG and HHT axes.

Role of gonad or thyroid ?