CHAPTER - I

SEASONAL HISTOMORPHOLOGICAL ALTERATIONS IN THE GONADS, ADRENALS AND THYROID IN NORMAL AND PINEALECTOMISED DOMESTIC PIGEONS, COLUMBA LIVIA

The first demonstration of pineal's physiological role in reproductive system was the observation of precocious sexual maturation in a six year old boy whose pineal was destroyed by a tumour (Heubner, 1398). This gave the initial impetus to direct more studies on understanding the regulatory influence of pineal in the activities of the gonad. Most of these early studies used the classic approach befitting that of simple secretory glands without taking into account the neural and environmental factors and yielded many conflicting results. These conflicting results and observations, and that the human pineal calcifies post-puberty, dampened the early enthusiasm and precluded further studies. Subsequently it was, nevertheless, shown that the calcified pineal is still an active structure (Wurtman et al., 1964). Renewed interest on pineal in the last two decades has ushered in a new era of pinealology during which the relationship between pineal and reproductive physiology has been increasingly documented and elucidated. The predominant influence of the pineal gland on reproduction that has been recognised is one of inhibition. Though a number of potential pineal hormones have been isolated, melatonin the principal indolamine hormone has been shown to have the inhibitory effects

on gonads (Wurtman, 1973; Cardinali, 1974). The other potent pineal principle structurally identified and also having antigonadal abilities is the polypeptide, arginine vasotocim (AVT) (Vaughan <u>et al</u>., 1976).

Though there is no concensus as yet about the antigonadotrophic principle of the pineal, either melatonin or AVT or both may be considered to have definite antigonadal properties. The antigonadal properties of pineal principle(s) though initially identified in mammals (as well exemplified in hamsters); such actions have also been observed in birds (domesticated species) and some lizards (Pitis et al., 1970; Levey, 1973; Saxena et al., 1979; Johnston and Zucker, 1980; Haldar and Thapliyal, 1981; Peter et al., 1981; Pevet et al., 1981). Paradoxically, this property does not seem to be universal and actioms ranging from progonadal to, antigonadal or even no influence have all been reported in mammals, birds and fishes (Stalsberg, 1965; Sayler and Wolfson, 1967, 1968; Pitis et al., 1970; Joss, 1973; Saxena et al., 1979; Johnston and Zucker, 1980). In majority of the cases a definite pineal involvement in reproductive activities has been realised and in most of them interaction with seasonal environmental cues (mainly photoperiod and temperature) eithe $_{f r}$ in tandem or in series is recognised. Whereas short photoperiod induced gonadal regression in hamster and rats is mediated by the pineal, long photoperiod induced gonadal activation in ewes is also under the influence of the pineal. Similarly in birds

too, there are differing reports on the involvement of pineal in the various facets of reproductive activities (Stalsberg, 1965; Sayler and Wolfson, 1967, 1968; Saxena <u>et al.</u>, 1979). 15

It is these differing pineal involvement in birds, that had prompted studies on wild pigeons in this laboratory. Since tropical species do not seem to use photoperiod as a definite cue to regulate their breeding activities, the experiments were conducted under natural photoperiodic conditions. These studies conducted during recrudescent, breeding and regression phases have indicated a definite progonadal role of pineal in wild pigeons; as marked by gonadal regression post-pinealectomy during recrudescent and breeding periods, and no effect in the non-breeding period (Ramachandran et al., 1984). In this context it was thought interesting to study the possible influence of pineal in the domestic pigeons as there are reports indicating antigonadal action of pineal in domesticated species of birds. The present study on the effect of pinealectomy on the histomorphology and gonado-somatic index was conducted during both breeding and non-breeding periods. Since adrenals and thyroid too are implicated in reproductive activities and as pineal is suspected to have some, as yet imprecise, interaction with these endocrine organs, histomorphology and histosomatic indexc of these glands were also studied under the pinealectomised ·) (*) condition.

MATERIAL AND METHODS

Adult domestic pigeons in the weight range 250-300 gms procured from the local animal dealer were used for the experiment. The birds were maintained in the aviary with adequate food of grains and water <u>ad libitum</u> and were permitted a sufficient enough period of acclimation prior to the experimentation. A total of ninety pigeons of both sexes whose weights were recorded were then divided into three groups of thirty each (15 males and 15 females). Group I - birds were subjected to pinealectomy as per the method developed in this laboratory (Ramachandran <u>et al.</u>, 1984) and served as the experimental group (PX). Group II - birds were sham operated and served as the sham control (PN), and Group III - birds were intact ones which served as the normal controls (C).

Ten birds (5 males and 5 females) from each group were then sacrificed under mild anesthesia at intervals corresponding to 30, 45 and 60 days post-pinealectomy/post-sham operation and the weights of gonads, adrenals and thyroid were recorded. Prior to sacrifice, the body weight of the birds were recorded. After weighing, the tissues were fixed in Bouin's flud and processed in the routine fashion for histological studies. Paraffin sections of 5µ thickness were cut and stained with haematoxylineosin. The above studies were carried out during both breeding months (March-May) as well as non-breeding months (June - August). The absence of pineal in all pinealectomised pigeons was confirmed by examining the dissected brain of sacrificed birds.

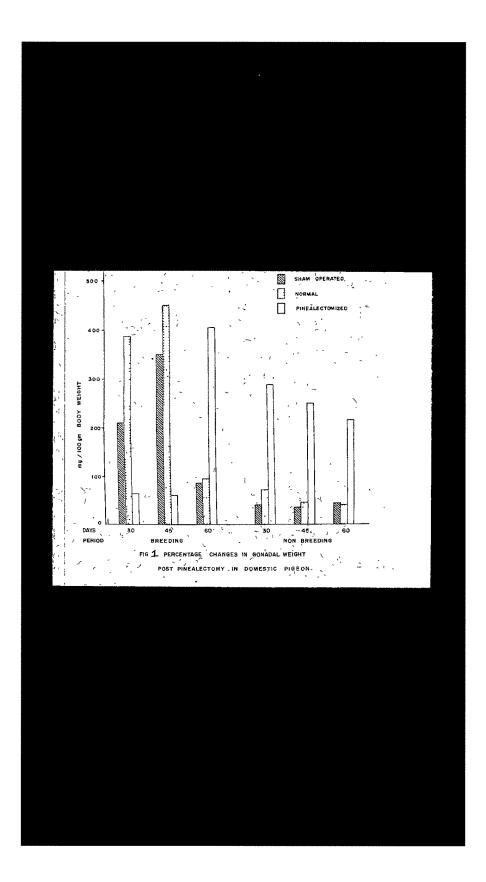
RESULTS

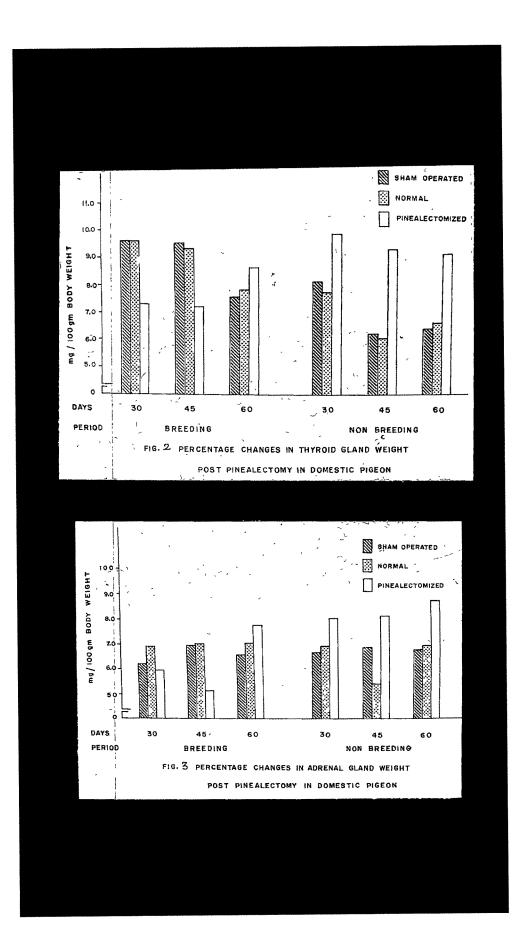
Changes in the relative weights of all the three organs (gonads, adrenals and thyroid) in the three groups of birds are represented in table 1 and figures 1-3.

SEASONAL VARIATIONSIN NORMAL BIRDS

The gonads and thyroid seem to show parallel changes in terms of relative weights, as well as their activity, assessed in terms of their histological appearance. Both the organs showed increased weight during the breeding season and reduced weight during the non-breeding season. In contrast, the adrenal weight was not altered much during the two seasons, though alterations in histological profile in terms of cortical to medullary activity were very much in evidence.

Histologically the gonads in the breeding season showed well developed spematogenically active seminiferous tubules and secretory Leydig cells in the case of testis, and multiple follicles in various stages of development and also many mature follicles and ovulated follicles in the case of ovary (Figs.46,416,18,29)





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Figs. 4a and b : Histological picture of testis of control birds during early breeding period. 160 X Note the intense spermatogenic activity with bundles of sperms in the tubular lumen.
Figs. 5a,b and c : Histological profile of testis of PX birds after 30 days in the early breeding season. 160 X
Note the shrunken tubules and various degree of germ cell

damage.

SB - Sperm bundles ; ST - Spermatids.

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Figs.6	i a	and	þ	:	Histological appearance of testig. of control birds during the mid breeding season showing tubules with active spermatogenesis. 160 X
Figs 6	i a	and	Ь	:	Histological appearance of the testis of PX birds after 45 days in the mid breeding season. 160 X
,					Note the highly regressed tubules with damaged and necrotic germ cells.

SB - Sperm bundles ; ST - Spermatids

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Figs. 8a,b and c : Histological picture of testis of control birds in the late breeding period showing regressed tubules. 160 X

Figs. 9a,b and c : Histological appearance of testis of PX birds after 60 days in the late breeding period. 160 X Note the enlarged tubules with the reinitiated spermatogenesis.

SB - Sperm bundles ; ST - Spermatids

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Figs. 10a,b and c : Histological picture of testis of comtrol birds in the nonbreeding period. 160 X

Note the varying degrees of germ cell damage in the inactive tubules.

Figs. 11a,b and c : Histological picture of testis of PX birds after 30 days in the non-breeding period.160 X

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Note the enlarged tubules with the various degrees of spermatogenic reactivation.

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SB - Sperm bundles ; ST - Spermatids.

Figs. 12a and b : Histological picture of testis of control birds in the nonbreeding period.' 160 X

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Note the highly regressed tubules with marked hyalization of basal membrane and degenerated germ cells.

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Figs. 13a and b : Histological picture of testis of PX birds after 45 days in the non-breeding period showing spermatogenically active seminiferous tubules. 160 X

SB - Sperm bundles; ST - Spermatids

Fig.: 14 :

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Histological picture of testis of control birds in the nonbreeding period showing lipid laden cells and spermatogenically disrupted tubules. 160 X

Figs.15a and b: Histological picture of testis of PX birds after 60 days showing reactivation of spermatogenesis to various degrees. 160 X

SB - Sperm bundles ST - Spermatids.

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Figs.16a and b	;	Histological picture of ovary of control birds during early breeding period. 160 X
		Note the presence of follicles in various stages of development.

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Figs.17a,b and C: Histological picture of ovary of PX birds after 30 days in the early breeding period.160 X

Note the follicular atresia.

AF - Atretic follicle; APF - Atretic primary follicle; APOF - Atretic post-ovulatory follicle; G - Granulosa; PF - Primary follicle; TE - Theca externa; TI - Theca interna;

Figs.18a and b : Histological picture of ovary of control birds during mid breeding period showing active folliculogenesis. 160 X

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Figs.19a and b : Histological picture of ovary of PX birds 'after 45' days in the mid breeding period. 160 X

Note the atretic changes in the follicles.

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AF - Atretic follicle; G - Granulosa; MF - Maturing follicle; PF - Primary follicle; T - Theca.

۰ ۱	EXPLANATIONS TO FIGURES
Figs.20 and 21 :	Histological picture of ovary of control and PX birds (after 60 days) during late breeding period. 160 X.
	Note the comparatively well developed active follicles in Fig.21.
AF - Atretic fol	licle; G - Granulosa ;

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MtF - Mature follicle; TE - Theca externa;

TI - Theca interna.

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PX birds . after 30 days in the non-breeding season showing reactivation of follicular development. 160 X

AF - Atretic follicle ;APF - Atretic primary follicleG - GranulosaMF - Maturing follicle

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Figs.28a,b and c: Histological picture of thyroid of control birds during early,mid and late breeding phases. 240 X Note the gradual shrinkage of the follicles.

Figs.29a, b and c: Histological picture of thyroid of PX birds after 30, 45 and 60 days in the breeding season. 240 X Note the inactive follicles in Fig.29a and enlarged active follicles in 29c.

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J	:	-	EXPLANATIONS TO FIGURES	
	Figs.30a,b	and c	: Histological picture of thyroid of control birds in the non-breeding season showing reduced inactive follicles. 240 X	
	Figs.31a,b	and c	: Histological picture of thyroid of PX birds after 30,45 and 60 days in the non-breeding season. 240 X	
			Note the enlarged active follicles.	
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Figs. 32a,b and c : Histological picture of adrenal of control birds during early, mid and late breeding seasons. 240 X

Note the gradual cortical regression and medullary activation.

Figs.33a,b and c :

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Histological picture of adrenals of PX birds after 30, 45 and 60 days in the breeding season: 240 X

Note the increased medullary activity in 33a and increased cortical activity in 33c.

C - cortex ; M - Medulla.

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Figs.34a,b and c : Histological picture of adrenals of control birds during non-breeding period. 240 X

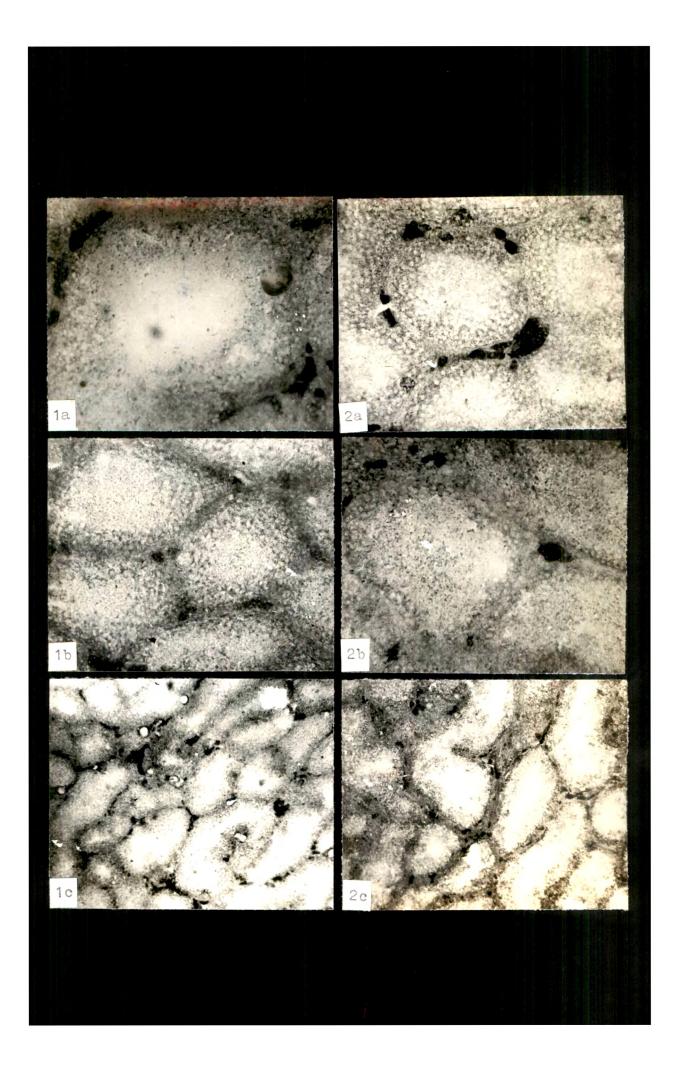
Note the reduced cortico-medullary ratio.

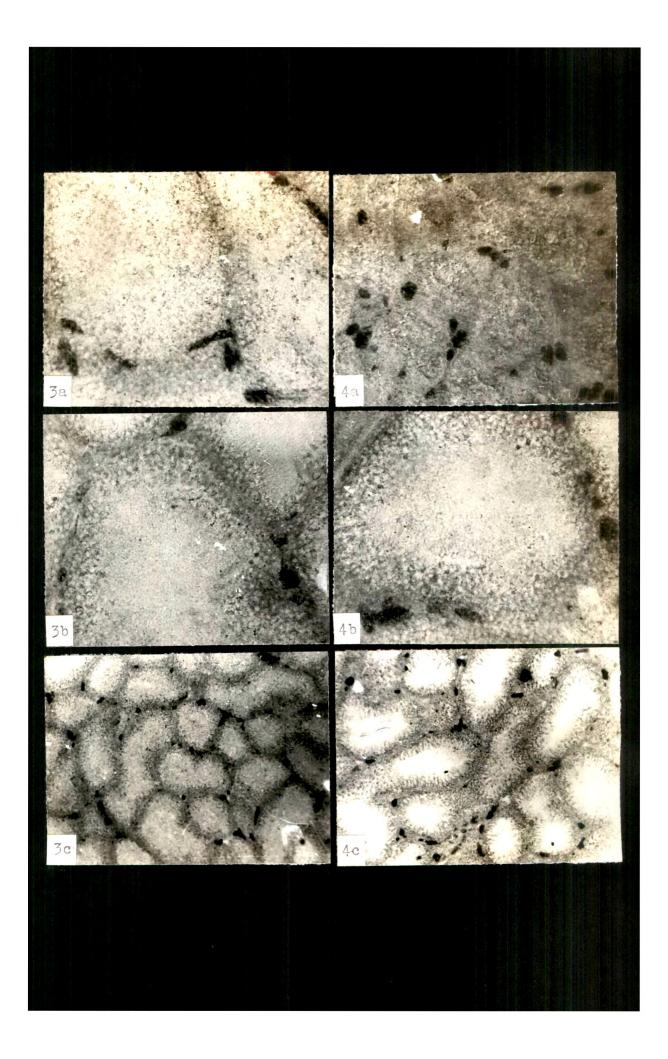
Figs.35a,b and c : Histological picture of adrenals of PX birds after 30, 45 and 60 days in the non-breeding period. 240 X

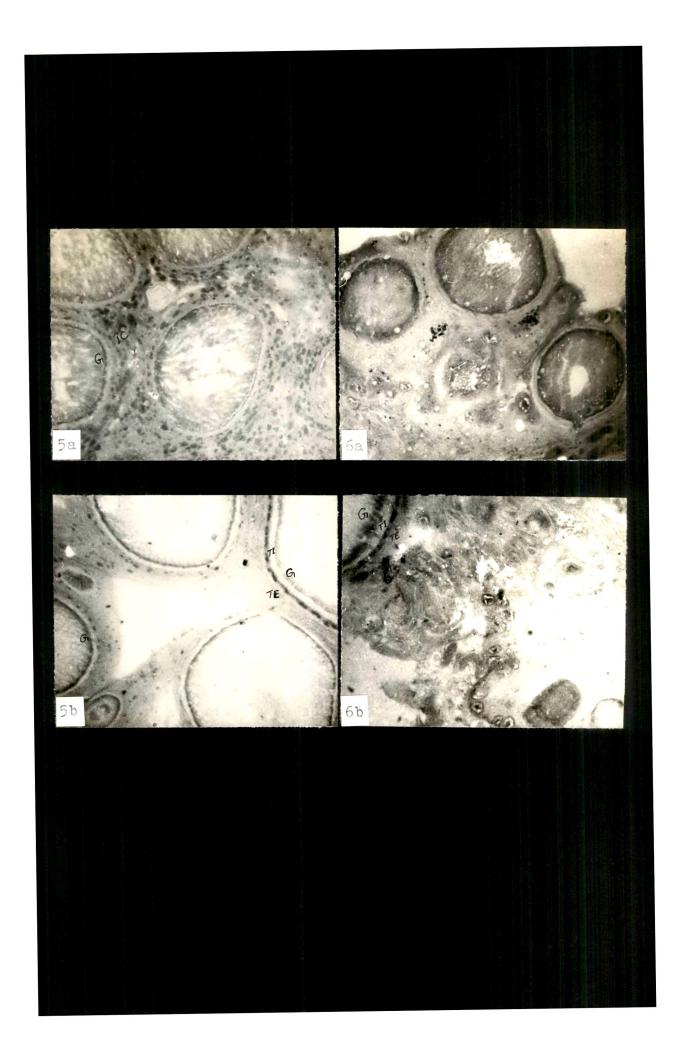
Note the increased cortico-medullary ratio.

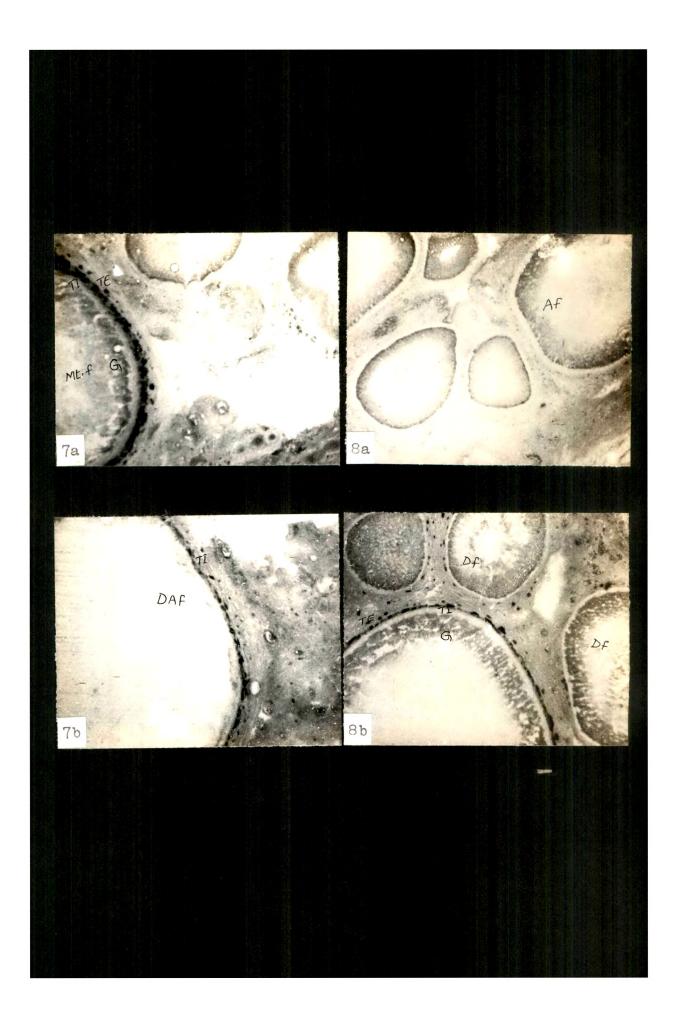
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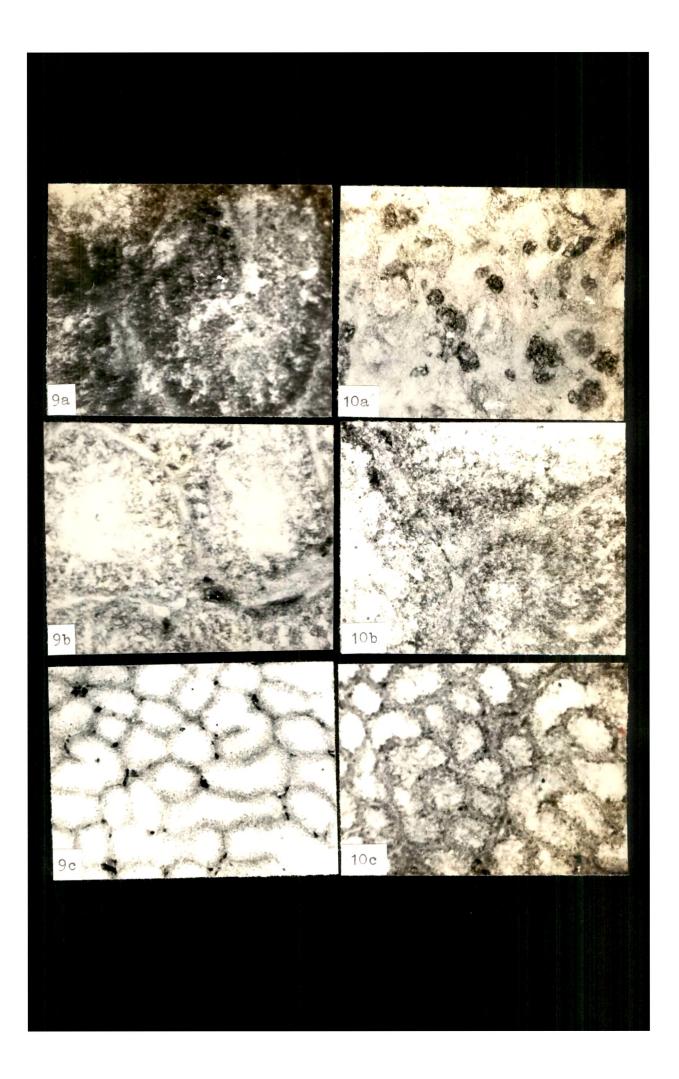
C - Cortex ; M - Medulla.

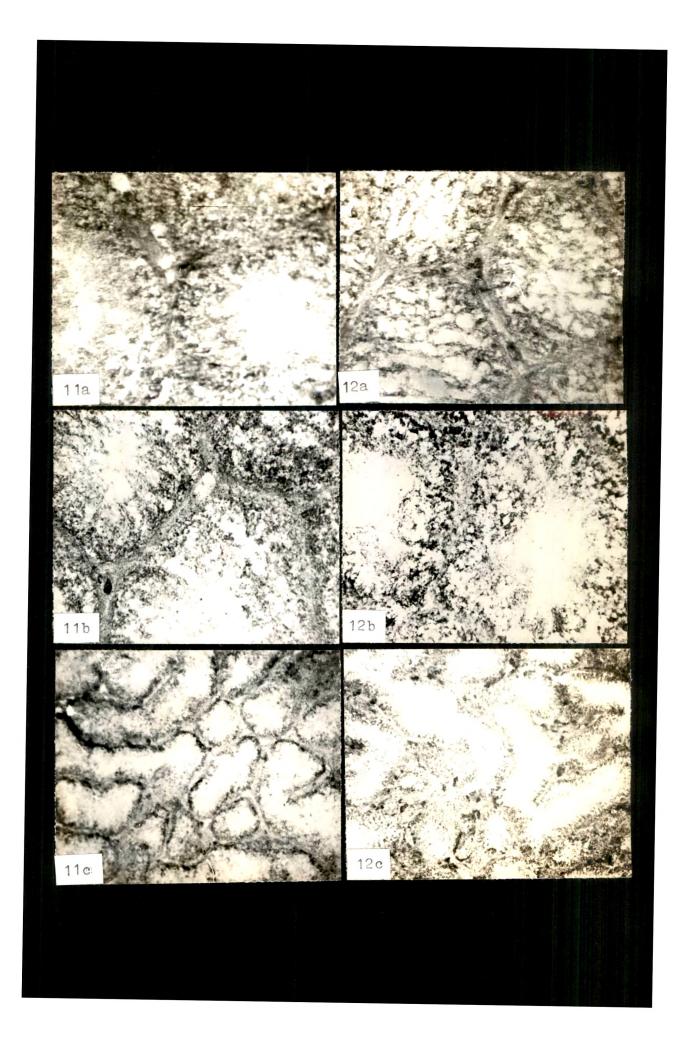


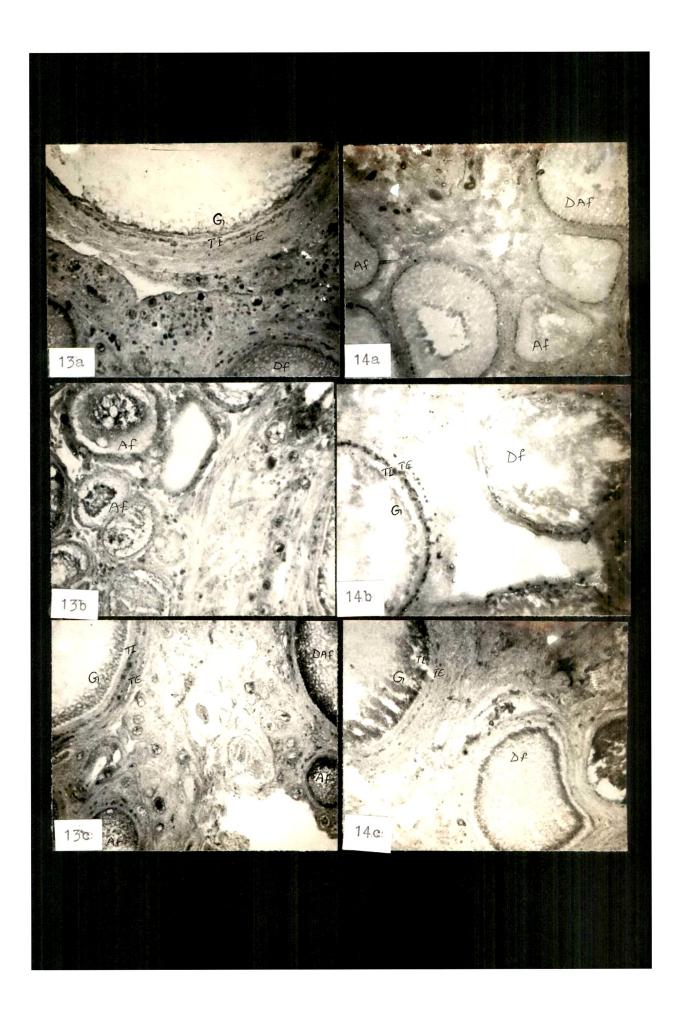


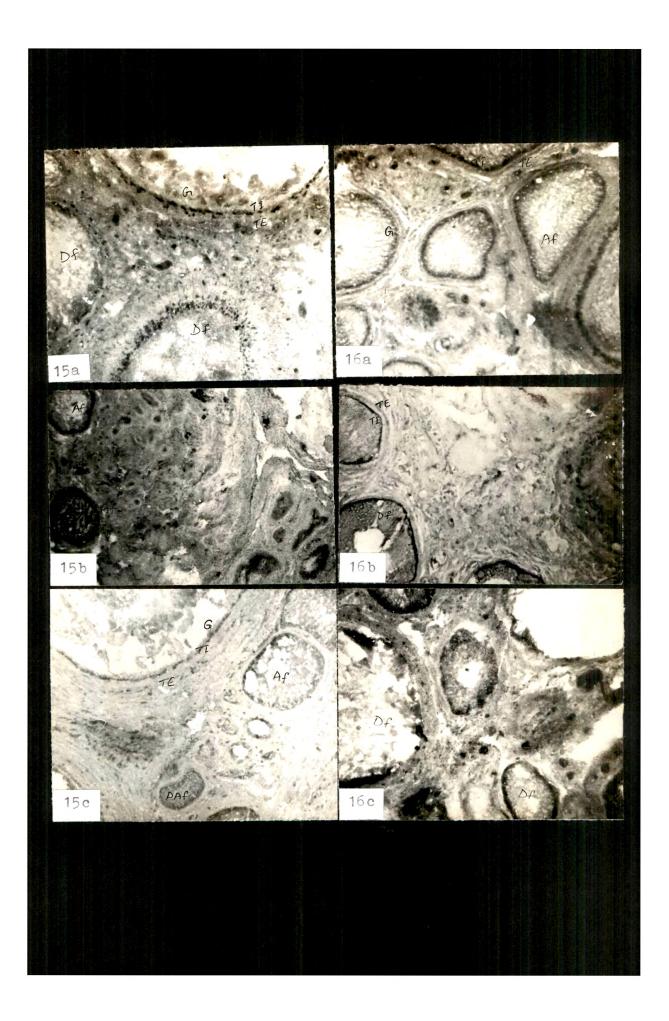


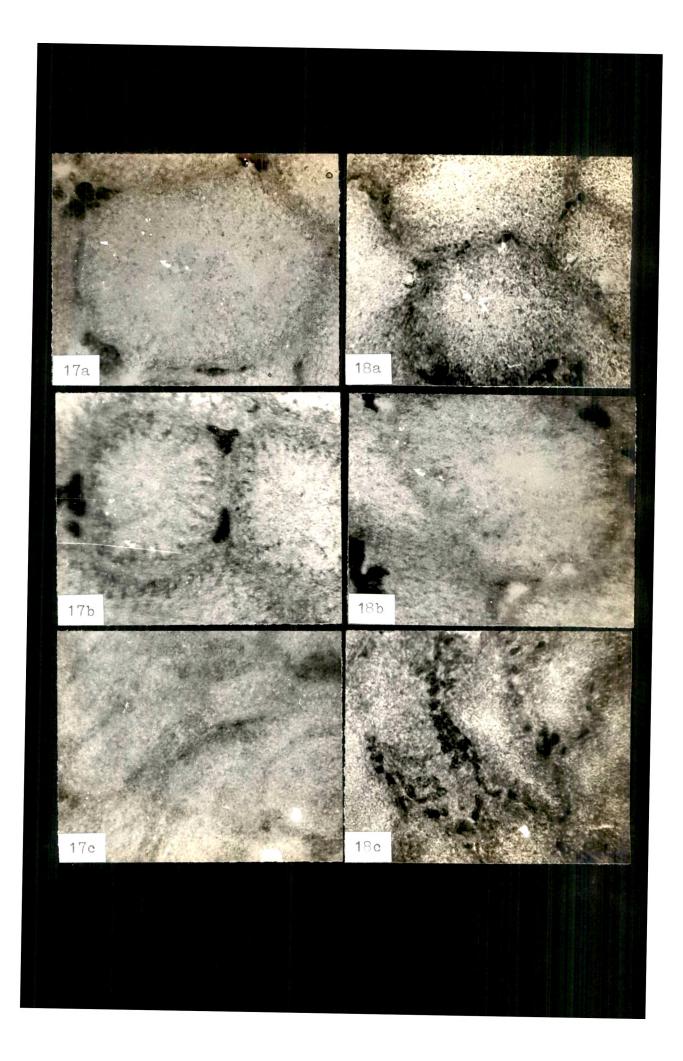


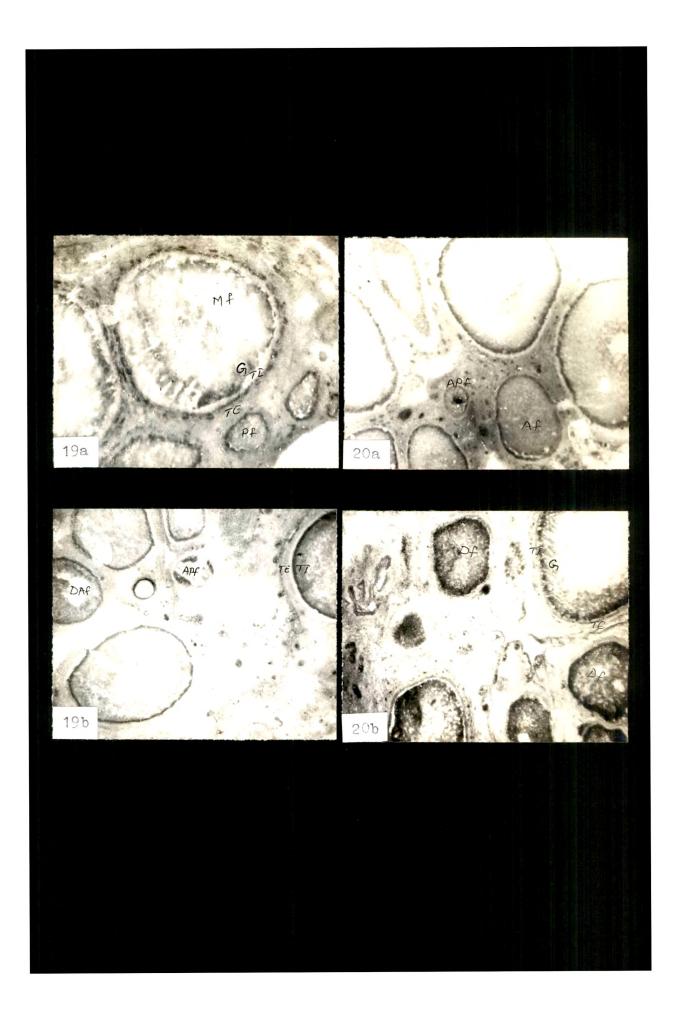


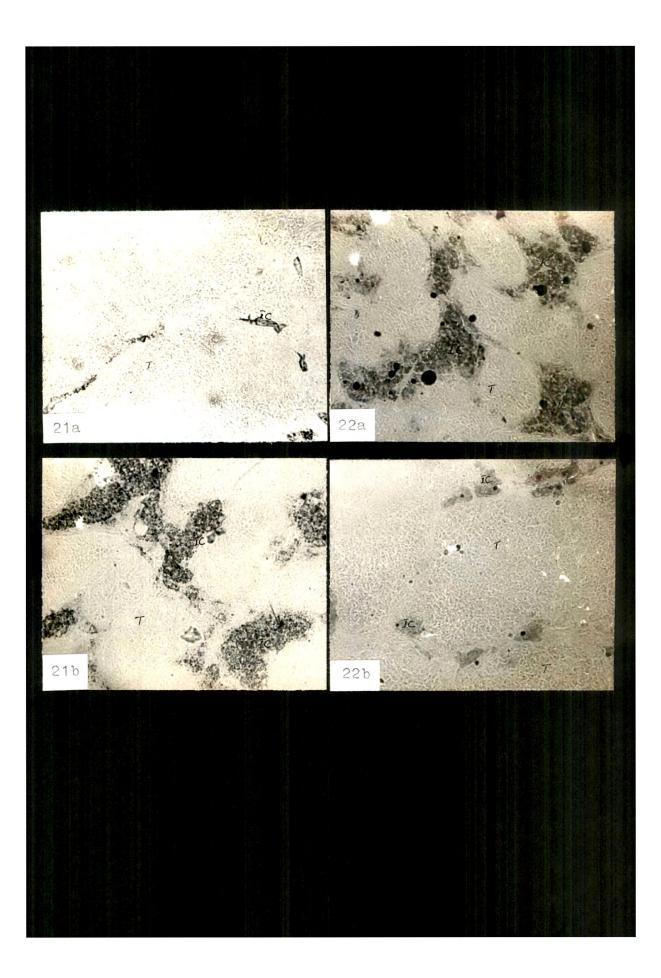


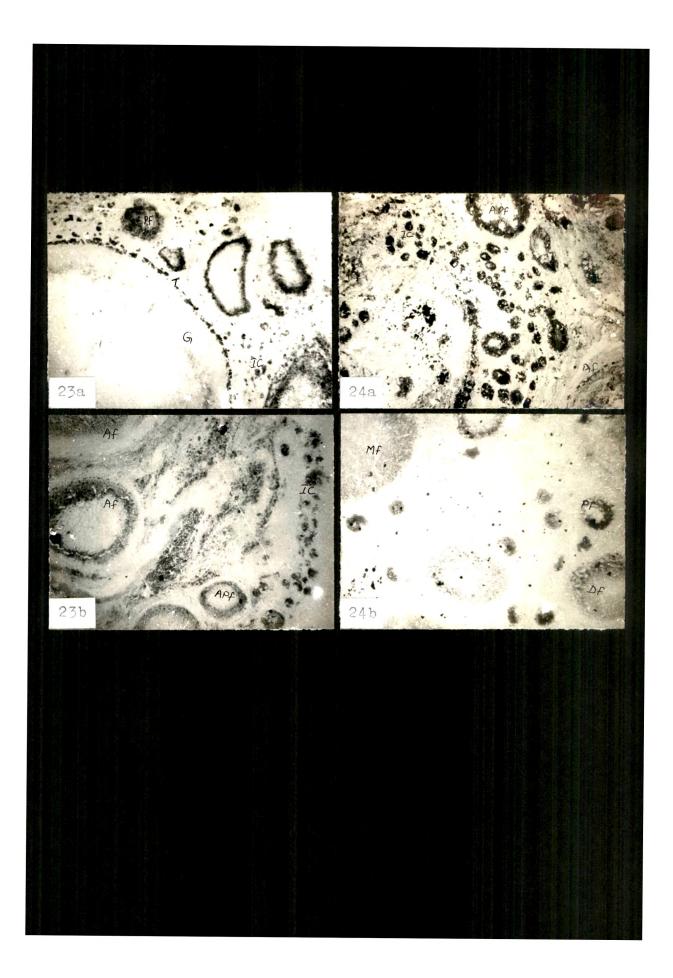


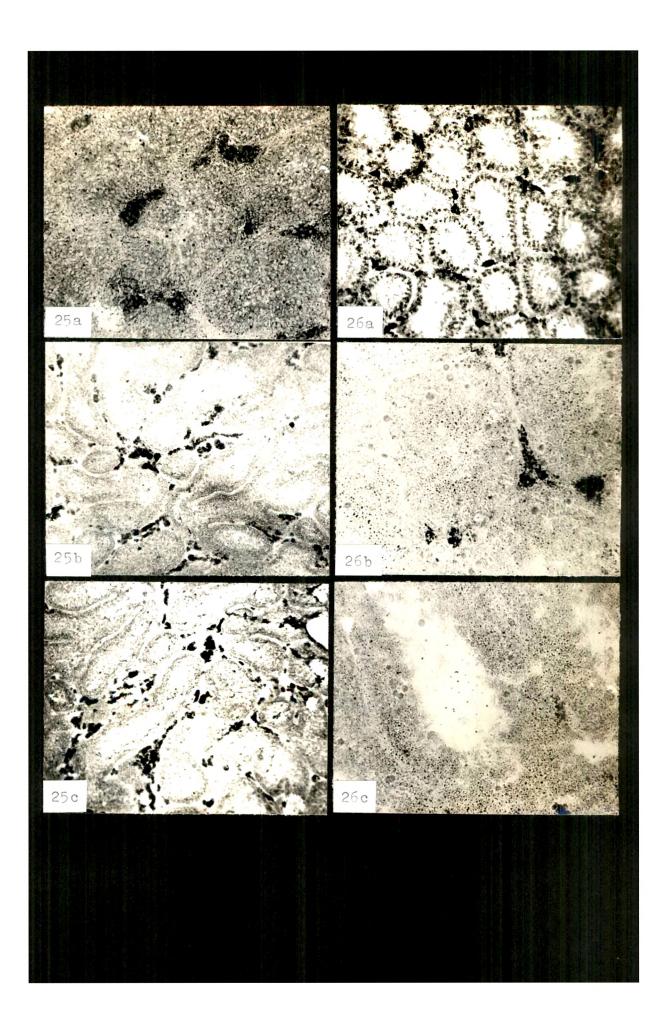


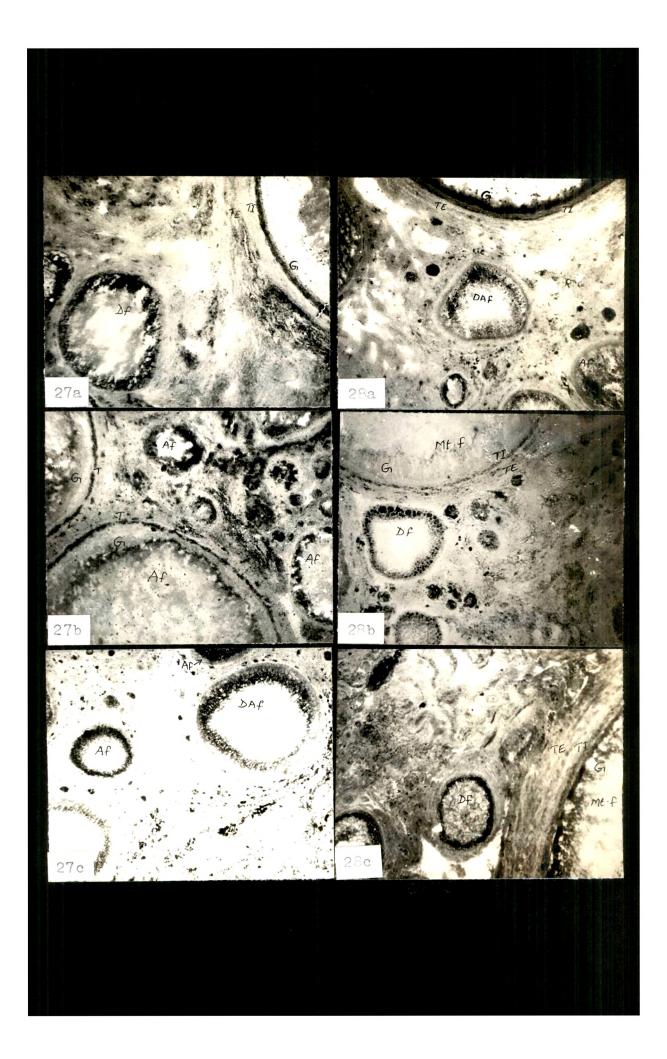


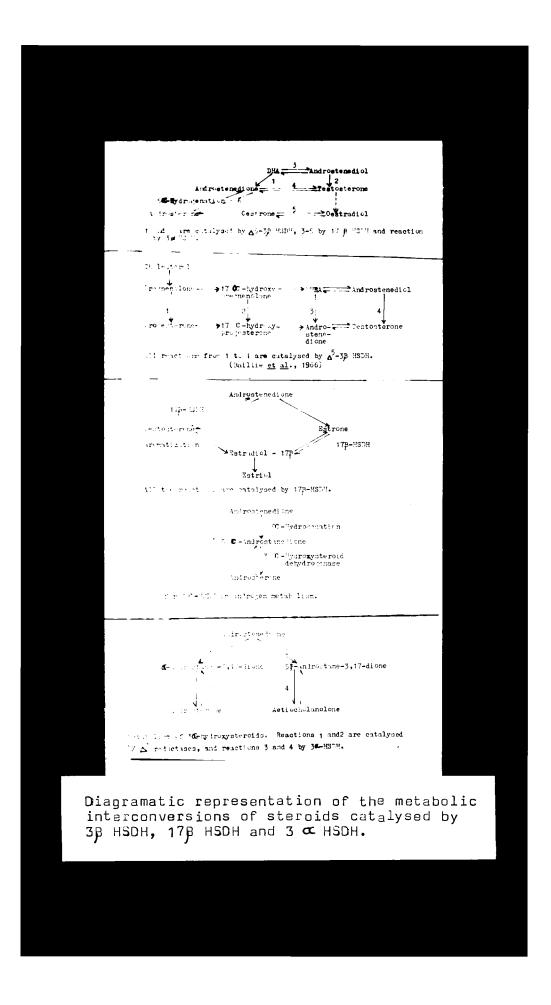


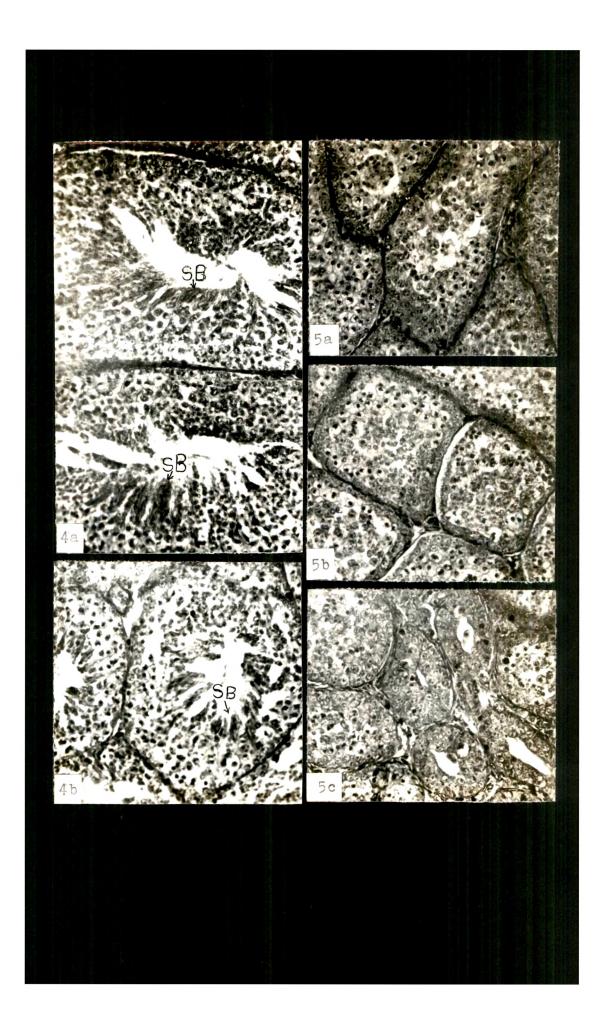


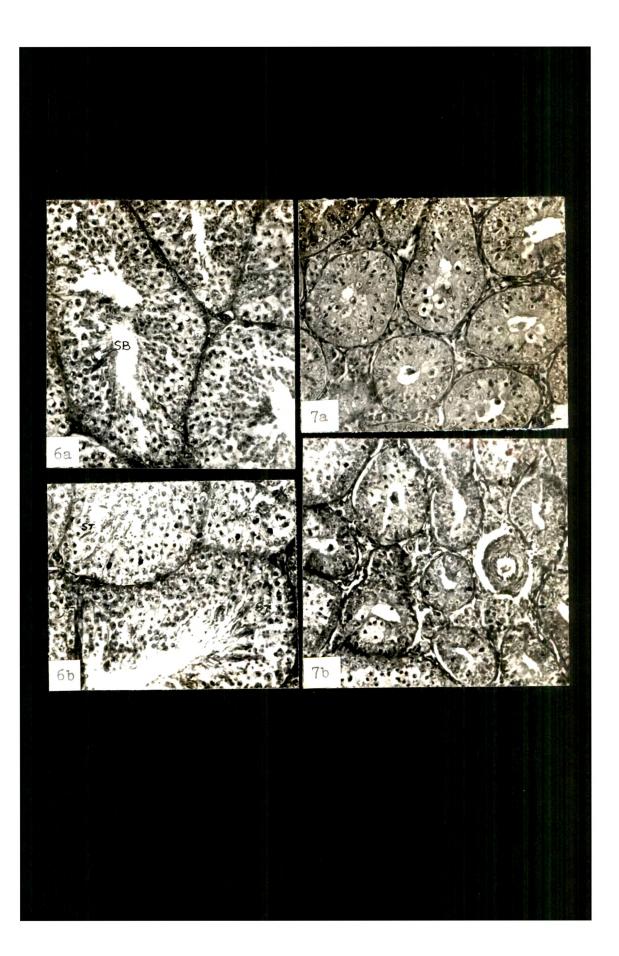


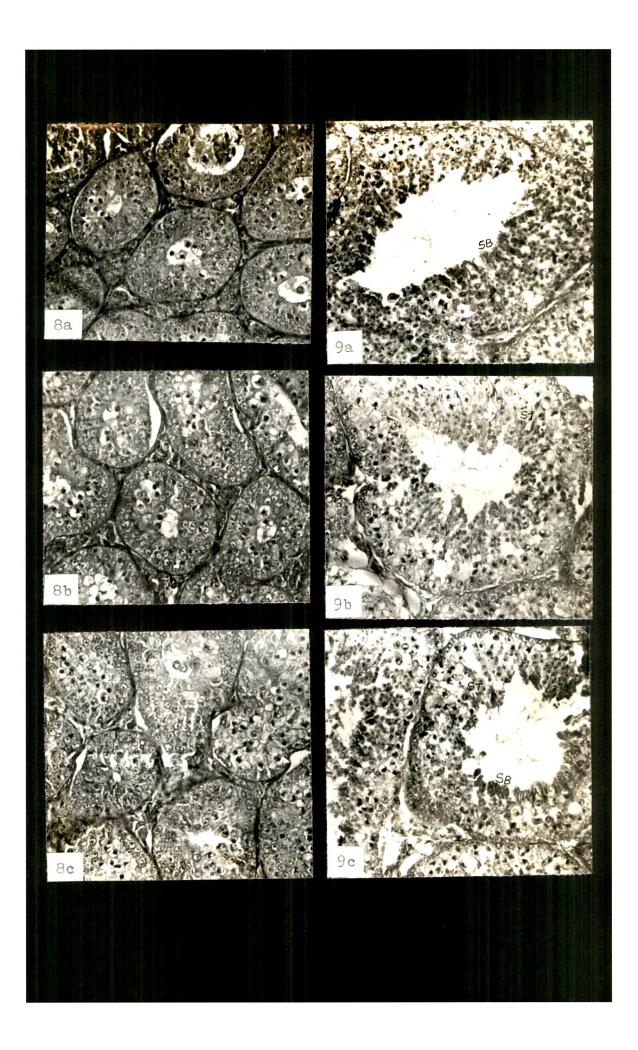


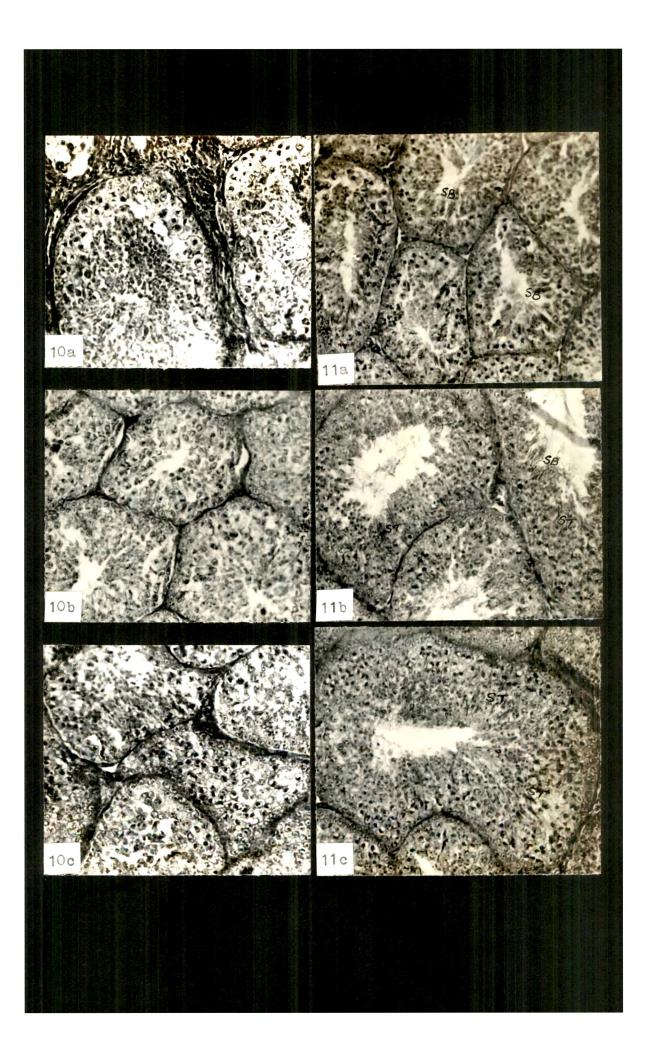


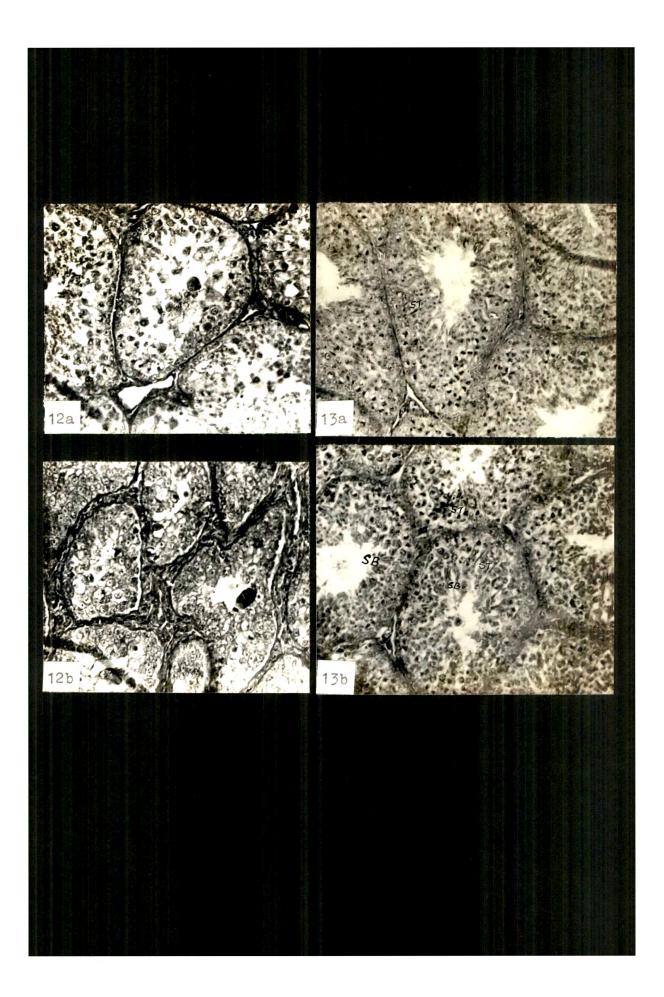


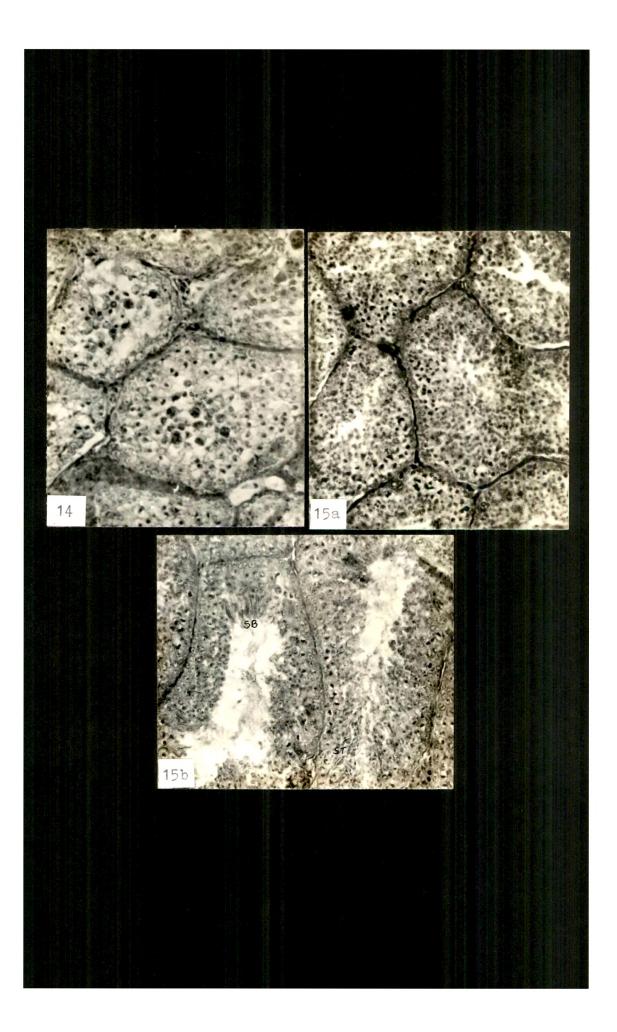


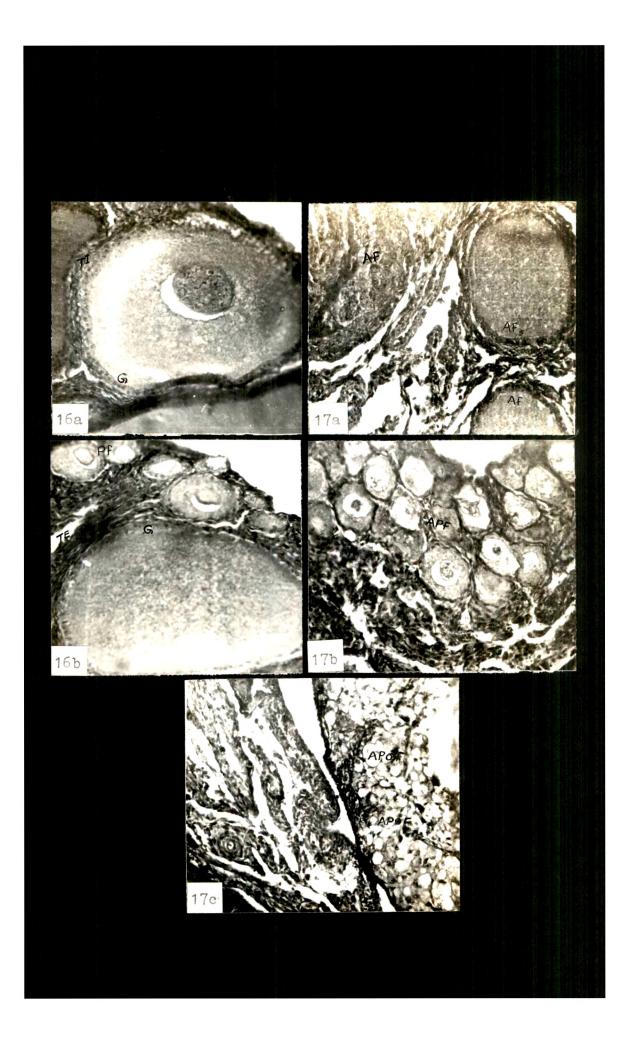


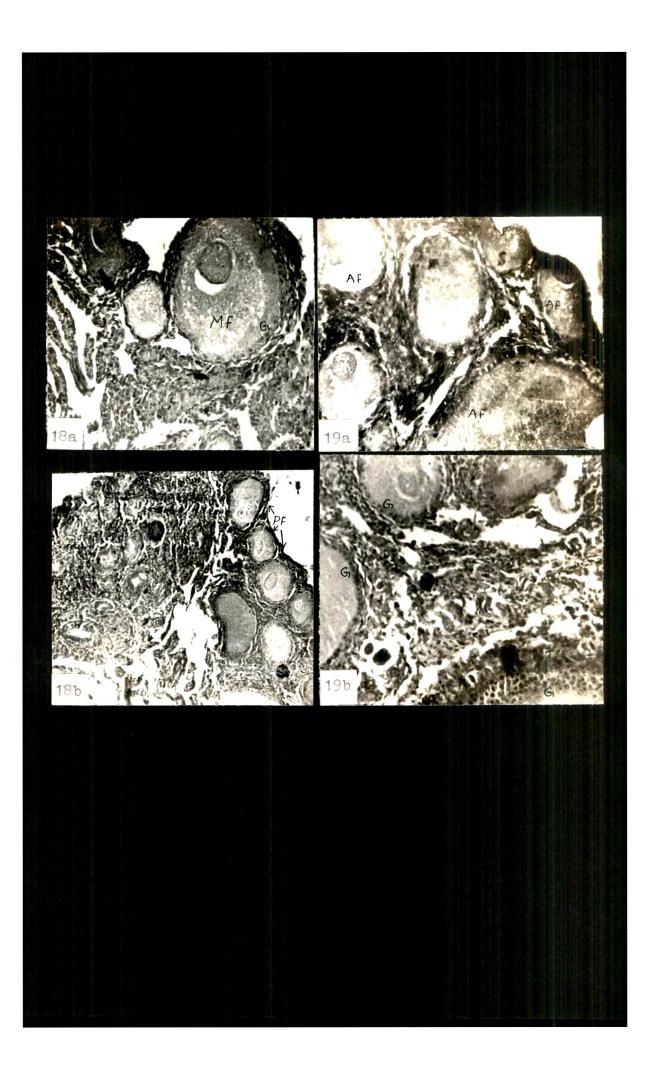




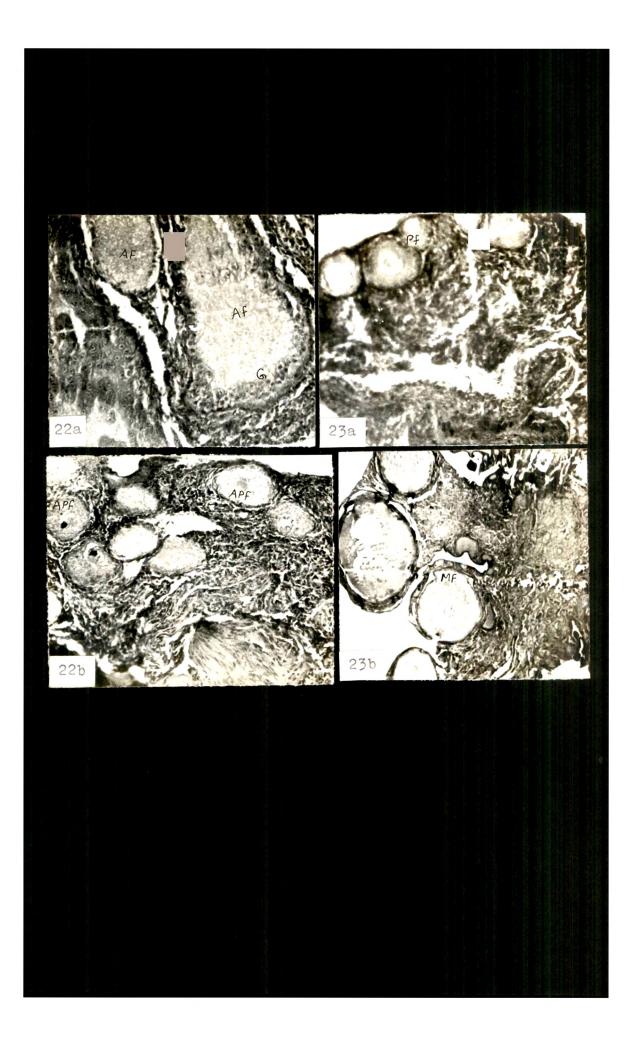


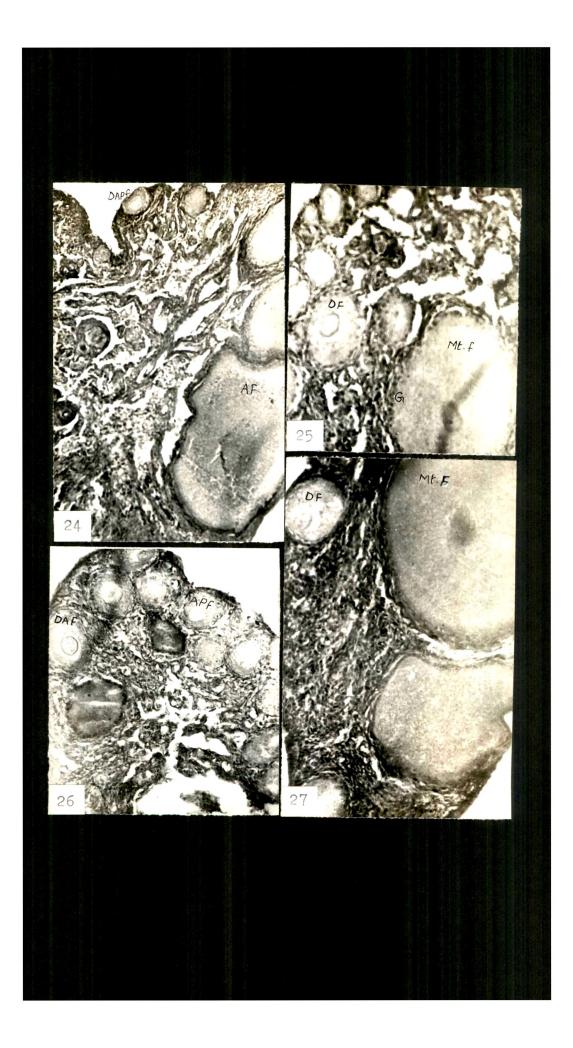


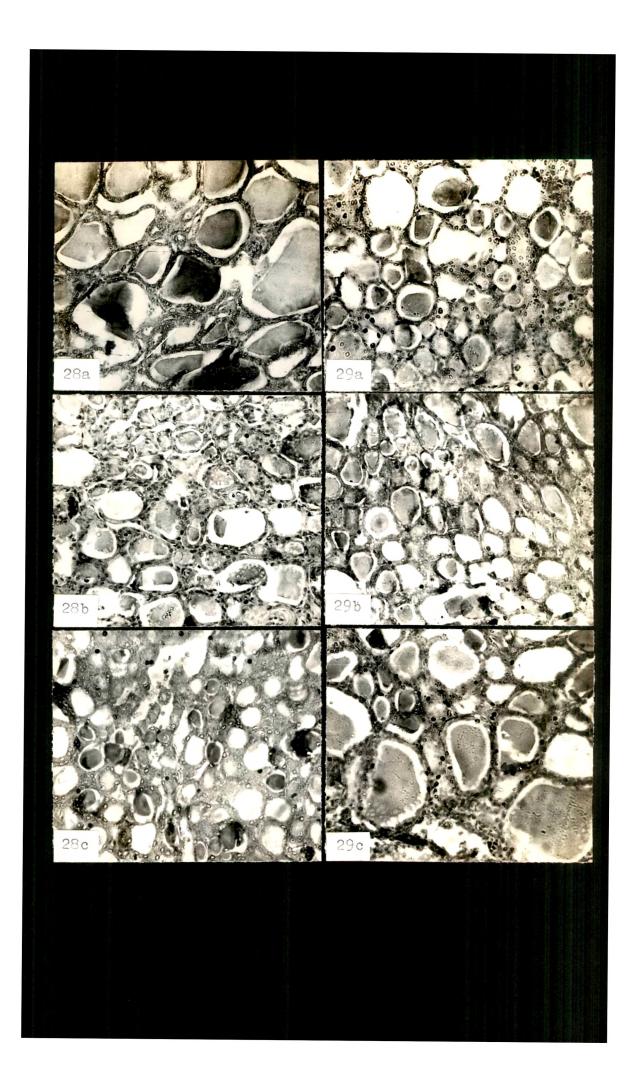


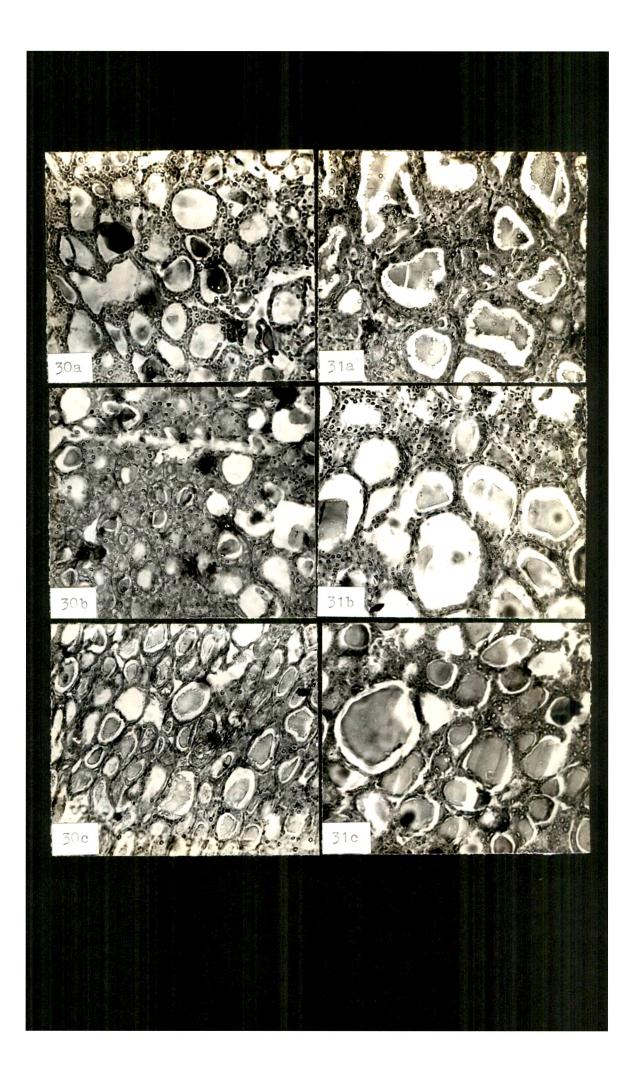


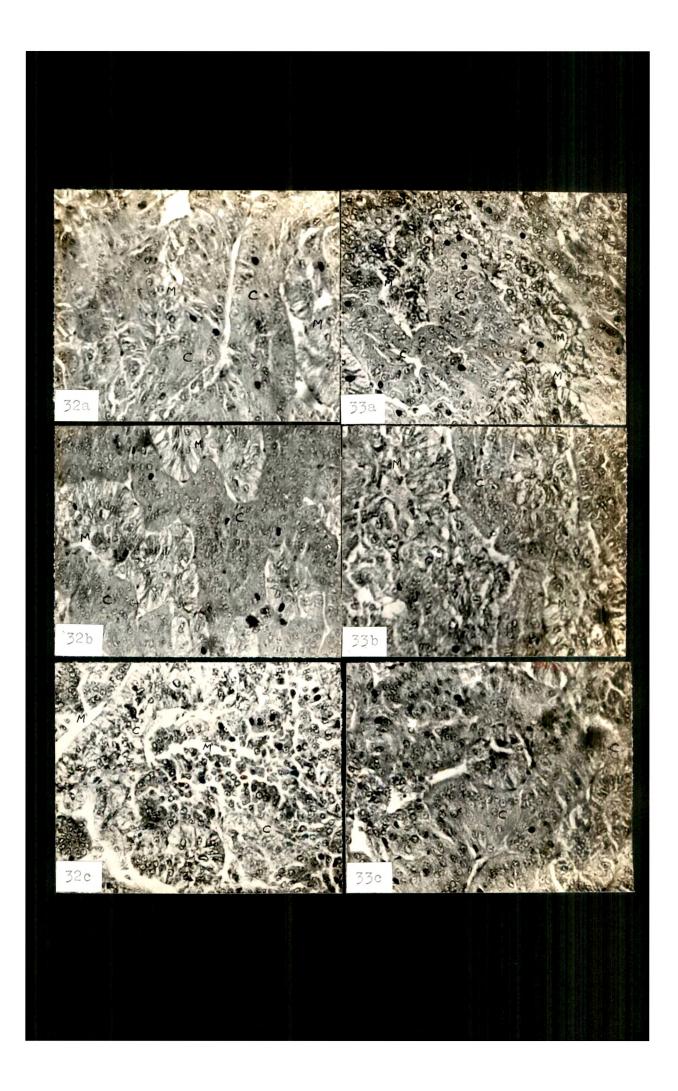


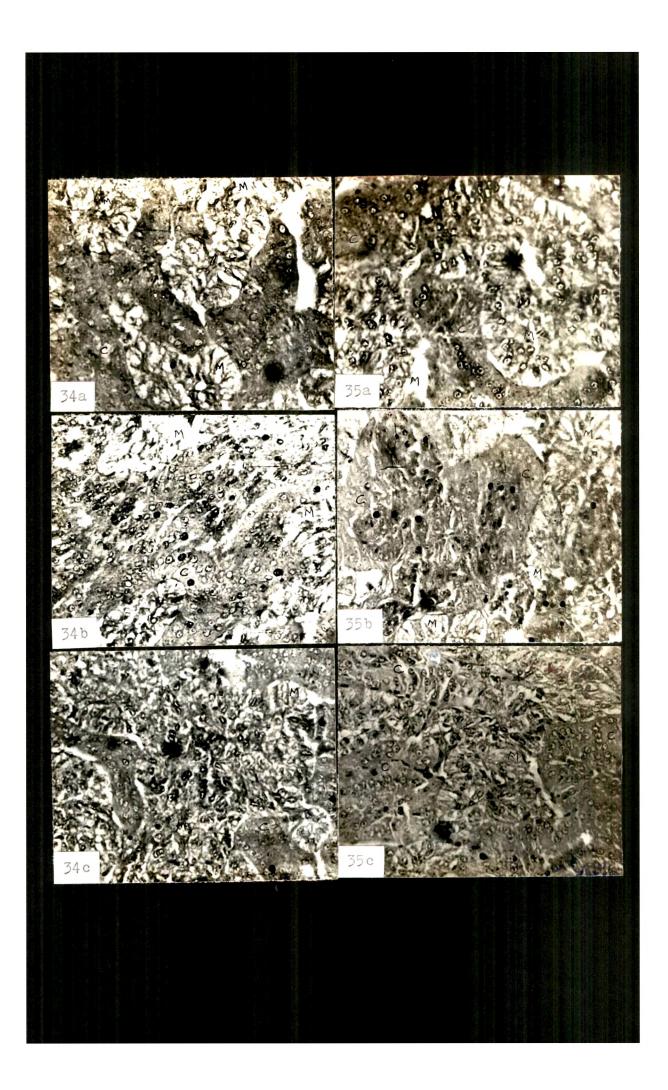












Corresponding observations on thyroid and adrenal indicated enlarged thyroid follicles lined by cells of increased height, and predominant and active adrenocortical cell columns. Many of the thyroid follicles were empty of their colloidal content while other follicles were with varying quantity of colloidal material. Increased cortical/medullary ratio could be easily discerned in the adrenal sections (Figs $2g \neq 32$). The non-breeding period was marked by regressed seminiferous tubules which were spermatogenically inactive in the testis, and numerous small primary follicles and abretic follicles in the ovary. The adrenal was marked by regressed cortical tissue and hyperactive medulla while in the thyroid, the follicles were reduced in size with reduced cell height and accumulated colloid (Figs.40,12,14,22,24,26,30,434)

SEASONAL VARIATIONS IN THE PINEALECTOMISED BIRDS

The alterations induced by pinealectomy on the relative weight and histological profile of the three organs were of a differential nature in the two seasons. Whereas the weights of the three organs decreased significantly in the breeding season, the weights were increased during the non-breeding period. The increase in weight post-pinealectomy was very much in evidence by the late breeding period itself. These changes in organ weights are depicted in Table-1. Histological observations revealed smaller collapsed seminiferous tubules in the testis and many developmentally arrested follicles in the ovary during

19-821 the breeding season (Fig. 57,9,17,). Many of the tubules were lined by either single or double layered hypertrophied cells with accumulated cell debris in the lumen. Follicular atresia was a common feature in the ovary of PX birds. However, during the non-breeding period the seminiferous tubules were enlarged and active spermatogenesis could be discerned within them (Figs. 11, 13 # 15). In the ovary there was renewed folliculogenesis (Figs. 23,254.27) which was not observable in the control birds. In the adrenals of PX birds the cortical/medullary ratio was visibly decreased and the hyperactive medullary component was the feature during the breeding period, while in the non-breeding period the condition was reversed with increased cortical/medullary ratio and active cortical cells, a condition similar to that of the adrenals of control birds in the breeding period (Figs. $33 \neq 35$). Similarly the thyroid too responded differentially with PX induced decreased follicular size with reduced cell height and accumulated colloid during the breeding season and enlarged follicles with increased cell height and depleting colloid content during the non-breeding period (Figs. 29 + 3).

DISCUSSION

Seasonally breeding animals including birds are characterised by cyclic changes in gonadal weight and activity. Endocrine glands such as the thyroid and adrenals which are implicated with various aspects of reproductive physiology could be considered to show alterations in structure in conjunction with the cyclicity of gonads. However, there are only a few studies on this line, much less so in birds. The present study in this light is pertinent and has shown definite alterations in adrenal and thyroid activity in conjunction with gonadal activation and regression. Apparently both the thyroid and adrenals seem to be actively involved in breeding activities as marked by their histological appearance. Increased adrenocortical activity and enlarged thyroid follicles with depleted colloid content in conjunction with active gonads in the breeding season are indicative of parallel adrenal-gonad and thyroidgonad axis in the tropical domestic pigeons. This is in contrast to the previous observations on wild pigeons where a parallel adrenal-gonad axis and an inverse thyroid-gomad axis were inferred (Ramachandran et al., 1984). The literature survey, on inter-relationship between gonadal activity and adrenal and thyroid in birds provide information favouring both parallel (Riddle, 1923) as well as inverse (Bhattacharya and Ghosh, 1965) relationships. Obviously these two endocrine structures have species specific interactions with the hypothalamo-hypophysiogonadal axis. Jallageas et al. (1978) and Bengt (1979) have reported inverse relationship between the adrenal and gonads of ducks and pied flycatcher respectively. Similarly with respect to thyroid activity too, both parallel (Chaturvedi and Thapliyal, 1980; Thaplial et al., 1982) and inverse (Thaplial and Panda, 1967; Jallageas and Assenmacher, 1974; Chandola and

Thapliyal, 1974; Gishi and Konishi, 1978; Jallageas <u>et al.,1978</u>), relationships have been reported. Jallageas and Assenmacher (1974) had also reported the inhibitory influence of both hypo and hyper thyroidic conditions on gonadal development. Since the breeding activities of birds to a greater extent are regulated by many internal and external cues which vary according to the adaptive requirements of the species concerned and as the adrenal and thyroid glands have pleotropic physiological effects, the possible adaptive interactions between these two endocrines and the hypothalomo-hypophysio-gonadal axis could be varied in nature. However, in the tropical domestic pigeons as observed in the present study, active gonadal functioning seems to be paralleled by increased thyroid and adrenal activities. This is further emphasized by the observed decreased activities of these two glands during the period of gonadal regression.

Though by far pineal's involvement in reproductive activities is well accepted, it is not universally singular. Available reports on pineal-gonad axis indicate a variable versatility espoused by this structure, and by serving as a link between the altered environmental conditions and the responses of the body it seems to modulate and regulate the reproductive activities to the optimum at the appropriate time. Majority of the studies in the form of pinealectomy or melatonin administration conducted on hamsters, and rats and mice have shown the gonadal regression induced by short

photoperiods to be mediated by the pineal (Hoffman and Reiter, 1965; Houssay et al., 1966a, 1966b; Motta et al., 1965; Reiter, 1968; Sandytino and Reiter, 1971; Reiter, 1978; Goldman et al., 1979; Reiter, 1980; Heath et al., 1981; Chen, 1981; Quay et al., 1982; David and Lynch, 1982; Jiri and Illnerova, 1982; Stetson et al., 1983). Similar antigonadal role of pineal has also been observed in birds and lizards by Shellabarger (1953), Kitay and Altschule (1954), Homma et al. (1967), Barfuss and Ellis (1971). Cuello et al. (1972), Halder and Thapliyal (1980), Misra and Thapliyal (1980) and Herbert (1981). In contrast to these reports are those demonstrating a progonadal action of pineal in mammals (Arendt et al., 1983), in birds (Sayler and Wolfson, 1967; 1968; McFarland et al., 1969; Balasubramanium and Subramanium 1979; Ramachandran et al., 1984) and in cyclostomes (Joss, 1973; Urasaki, 1973). Most of the above reports pertain to temperate: species of animals and the proximate cue involved is the photoperiod (long or short) which is interpreted in a favourable fashion by the pineal either in bring about gonadal activation or gonadal regression. Since the tropical species are not under the seasonal regulatory influence of photoperiod as a proximate factor in modulating reproductive activities, the present study conducted under natural photoperiod regimen has brought to light the dual role of pineal in domestic pigeons. The observed PX induced gonadal regression during the breeding period and activation during the non-breeding period to the tune of about 80-85 % demonstrate the progonadal and antigonadal roles of the

pineal in the two seasons. The histological profile of the gonads in PX pigeons during the two seasons bear ample testimony to the above. In all likelihood the pineal in domestic pigeon could be considered to produce two different principles, one with progonadotrophic properties during the breeding months and one with antigonadotrophic properties during the non-breeding months. Such an assumption seems realistic in the wake of the increasing repertoire of pineal associated substances that are being identified in the recent times. Incidentally, Saxena <u>et al.</u> (1979) had also cobserved similar progonadal and antigonadal actions of the pineal in the Indian weaver birds.

Apart from the pineal-gonad axis which is well established, even pineal-adrenal and pineal-thyroid axis too have gained interest in recent times. Since adrenal and thyroid are both known to show cyclic variations in activity in consomance with reproductive cyclicity, the current observations and the histomorphology of these two glands in the PX condition, can provide information on the possible indirect mode of action of pineal on gonadal structure and function apart from providing information on the direct pineal-adrenal and pineal-thyroid interactions. As noted in the present study, pinealectomy induced fall in weights and decreased activity of the glands in the breeding season. In the non-breeding period pinealectomy produced reverse set of changes. Apparently, pineal in domestic pigeons has a negative regulatory influence on both adrenal and thyroid.

Similar inhibitory influence of pineal on thyroid and adrenal glands has been illustrated by both melatonin injections and pinealectomy in mammals, birds and lizards (Ishibashi et al., 1966; Houssay et al., 1966b; Kinsen and Singer, 1967; Narang et al., 1967; Kinsen et al., 1967; Houssay and Pazo, 1968; Ogle and Kita (y, 1976; Jerry, 1978; Haldar and Thapliyal, 1979; Jerry et al., 1979; Cogburn and Harrison, 1980; Singh and Prasad, 1981; Jerry et al., 1982). All the above studies pertain to a particular period of the breeding cycle only while the present study was carried out during both breeding and nonbreeding periods. Accordingly differential responses have been obtained. Thyroid activity is enhanced after PX in the nonbreeding period, while it is reduced in the breeding period. This may indicate a parallel relationship between the thyroid and gonad in which pineal could have a regulatory influence. Similarly, adrenal too responded in a differential fashion with PX in the breeding season inducing reduced cortical activity and increased medullary activity and in the non-breeding season the reverse being true. What is apparent from the present results is the parallel adrenocortical-gonad activity and inverse adrenomedullary-gonad activity. The herein observed alterations in seasonal adrenal histology in control and pinealectomised 'animals suggest a tentative possibility of cortico-medullary activity ratio as a probable factor in controlling reproductive ' activity in domestic pigeons. This is however in contrast to the condition in wild pigeons were alterations in cortical activity alone seems to be involved (Ramachandran et al., 1984).