INTRODUCTION

The status of pineal in the endocrine orchestra has proceeded from a less popular enigmatic past to a very popular versatile present. The first clue to its glandular nature was provided by the observation of its extreme vascularity. Tilney and Warren (1919) and, Gladstone and Wakely (1940) were the first to advocate the idea that pineal had to have an internal secretory function. Interestingly, McCord and Allen (1917) published the first experimental observations on the physiological actions of bovine pineal extract. They observed that when fed to amphibians,the extract lightened their skin by causing the aggregation of the pigment granules in the melanocytes. This remained todate a pioneering milestone in pineal research and the factor responsible for this blanching effect was later identified as N-acetyl-5-methoxytryptamine or Melatonin (Learner et.al.,1958, 1959). Ironically, the scientific world generally ignored the pineal and denied it the status of an endocrine gland. One of the reasons for this is that the early experiments with pineal extracts either produced diametrically opposite effects or no effect at all (Engel, 1934; den Hartog Jager and Heil, 1935; Milcu , 1941). Similarly,variable results were also obtained in experiments involving pineal extirpation (Kitay and Altschule,1954; Thieblot and LeBars, 1955).Both the above reviews however tended to confirm an endocrine role for

pineal and suggested a controlling influence on reproductive organs. Over the last two and a half decades interest on pineal has gradually increased and has by now generated a volumious literature. Though the many scientific investigations during this period have had their own share of healthy controversies and disagreements, scientific thinking has started veering towards crystalized ideas of reproductive, endocrine and extra-endocrine effects of pineal. Based on the experiments carried out on non-mammalian vertebrates, the most clearly elucidated role of pineal is on diurnal chronobiological events like colour change, locomotor activity and temperature cycles (see Ralph, 1978; Gupta et al.,1979; see Underwood, 1989). The key to the ability of pineal to control many rhythmic cyclic processes like the above or even reproduction lies in its capacity to entrain and phase lock circadian rhythms with the result, even under constant conditions the rhythms persist and free run.

Bulk of the work in pinealology pertains to unravelling the functional sigificance of pineal in mammals. Major brunt of these investigations has focussed on the ability of pineal to influence the hypothalamo-hypophysial-gonadal axis. Though some of the earlier studies showed inconsistent and highly variable effects of pineal due to differences in the species,age of the animal, lighting

schedules, time of experimentation etc., further experiments performed under controlled conditions keeping in view the above factors have provided evidences for a generalised inhibitory role of the pineal in mammalian reproduction (Reiter et al.,1975; Johnson and Reiter, 1978; Bartness and Goldman, 1989). The pineal gland has been primarily recognised as a neuroendocrine transducer (Wurtman and Anton-Tay,1969) responding to photic and neural information by releasing pineal principles which in turn might act on brain centres, the hypothalamus, the pituitary and on the gonad. The most dramatic effect of pineal on the reproductive system is best documented in seasonally breeding mammals (see Ebels and Balemans, 1986; Tamarkin et al.,1976). Though melatonin is considered to be the most important pineal indole hormone, of late,even other indoles and peptides of pineal origin have also been shown to have physiological effects (Damian,1989; Vaughan et al.,1983).

Despite the impressive elucidations made in mammals, the role of pineal in avian reproduction has more or less remained enigmatic. Though its role in circadian functions like body temperature rhythms,perch-hopping, locomotor activity and even migratory restlessness have been more clearly shown (see Ralph,1978), the limited number of studies on pineal in relation to avian reproduction have produced such disparate results that it has become well

neigh impossible to make any meaningful conclusions (Ralph, 1978, 1981). The main reason for this frustrating situation is the isolated inadequate experimentations on limited species. Probably,more detailed and comprehensive investigations on a single species is needed to decipher the role of pineal in avian reproduction. 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 nonbreeding 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 nonbreeding 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 indicated a parallel pineal-adrenal-gonad axis and an inverse pineal-thyroid-gonad 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 is known to occur in many birds (see Chaturvedi,1993;see Thapliyal, 1993; Pathak and Chandola,1983).

However, to clearly elucidate the relationships between pineal, adrenal, thyroid and gonad, apart from pinealectomy, other experiments using pineal principles and related experiments are needed. It is in this background, that the present investigations were planned and executed. The part of the work embodied in the thesis has essentially attempted to seek specific answers to certain lines of thinking. Some of the queries raised in the first part of the thesis in relation to testicular functions are :

1. What is the effect of pineal indoles, specifically melatonin (M), methoxytryptophol (ML) and methoxytryptamine (MT) and also chemical pinealectomy by pCPA treatment on the testes during the breeding season ? Concurrently, their effects on the hypothalamo-hypophysial-thyroid (HHT) and hypothalamo-hypophysial-adrenal (HHA) axes have also been evaluated.
2. Can melatonin replacement to PX birds in breeding season prevent the effects of PX on the HHG, HHT and

HHA axes ?

3. What possible effects could exogenous melatonin have on these three axes in the nonbreeding season ?

Earlier studies from this laboratory on intact as well as PX birds on a seasonal basis had also revealed significant changes in carbohydrate metabolism involving tissue glycogen contents and plasma glucose levels (Patel et al., 1983,1988; Patel and Ramachandran, 1987; Ramachandran and Patel, 1987). Altered glucose tolerance and insulin sensitivity as well as tissue glucose uptake were also documented for PX pigeons (Ramachandran and Patel,1989). Though there are no studies showing influence of pineal on carbohydrate metabolism in birds, some sort of interrelationship between pineal and pancreas has been hinted at in mammals (Damian,1989; Gorray et al.,1979; Milcu et al.,1971; Notario, 1956; Petronio and Tavazza, 1958).This had provided the necessary impetus to simultaneously look into the possible changes in carbohydrate metabolism which may occur under the above mentioned experimental manipulations. In this respect, hepatic and muscle glycogen contents, hepatic phosphorylase and G-6-p'ase activity, plasma glucose level and pancreatic islet cell functions have been assessed concurrently.

It is very well known that many of the pineal

mediated functions are entrained by photoperiodic changes. This is clearly established with reference to long day and short day breeding mammals (Glass and Lynch,1981; Bittman et al.,1983). Importance of photoperiod in regulating the annual gonadal cyclicity of temperate species of birds needs no elaboration (Follet and Robinson, 1980; Follet, 1984;see Murton and Westwood, 1977; Nicholls, 1988). Even some of the tropical and subtropical species of birds are reported to show changes in reproductive functions in relation to photoperiodic changes (Maitra, 1986, 1987a,b; see Thapliyal and Gupta, 1989). A careful study of the seasonal changes in reproductive activity in the feral pigeons show that the annual gonadal recrudescence coincides with the winter solstice, while gonadal regression coincides with the summer solstice. This gave rise to a tentative idea that possibly the gradual but definite increase in day length following winter solstice may have some role in gonadal recrudescence and the decreasing daylengths subsequent to summer solstice may have some role in maintaining the regressed state of gonads. It is quite likely that these subtle changes in day lengths by altering pineal melatonin output may exert season specific actions on HHG, HHT and HHA axes. It was as a sequel to this line of thinking that seasonal investigations were deemed necessary. The second part of the thesis deals with some of the preliminary investigations carried out on this line. The first question posed in this

context was,

1. What would be the influence of a long photic schedule given towards the end of quiescent phase on gonadal recrudescence and HHT and HHA axes in intact and PX pigeons ?
2. What would be the effects of altered functional status of adrenal under the same conditions in intact pigeons?

Since there is very poor information base on metabolic changes in relation to photoperiodism and as adrenal is known to have metabolic functions, alterations in carbohydrate metabolism have also been evaluated under the above two experimental schedules.

CHAPTER I

ALTERATIONS IN THYROID HORMONE LEVELS AND HISTOMORPHOLOGY OF TESTIS, ADRENAL AND THYROID IN PIGEONS TREATED WITH PINEAL INDOLES AND p-CPA DURING THE RECRUDESCENT PHASE.

The pineal gland is by now more assuredly implicated as a modulator of reproductive process in mammals especially seasonal breeders (Hoffmann,1981; Reiter,1981,1982,1984). The reproductive effects of pineal is mediated principally by its methoxyindole hormone, Melatonin (M) and the best known action of M is its inhibitory effects on the reproductive system by way of its antigonadotrophic effect (Ralph,1978; Reiter,1980,1981; Blask,1981; Mess et al.,1981; Volrath,1981; Cardinalli et al.,1983; Chanda and Biswas,1988). Currently emerging mechanism of action of M on gonadotropin secretion is purportedly through its suppressive effect on the hypothalamic GnRH pulse generator (Silman,1991). Except for some minor difference, there is quite a bit of functional parallelism between the pineal of mammals and birds (Ralph,1981). This would suggest the pineal to have similar effects on reproductive functions in birds. However, the many different studies conducted on birds have failed to provide any convincing pan-avian species role for the pineal vis-a-vis reproduction (Ralph,1981). The limited number of species investigated

and inadequate experimental manipulations have precluded the emergence of any solid concept about the functional role of pineal in avian reproduction. Due to the varied and contradictory responses observed subsequent to pinealectomy (PX) or M administration (Ralph,1981; Meyer,1986), an understanding of the function of pineal in birds has till todate remained enigmatic.

Previous works from this laboratory on both domestic and wild pigeons have shown a progonadal role of pineal in the reproductively active phases as, PX in the recrudescent and breeding seasons resulted in gonadal involution (Ramachandran et al.,1987; Ramachandran and Patel,1988). Concurrent to PX-induced gonadal regression, alterations in the functional status of adrenal and thyroid were also observed alluding to definite pineal-adrenal and pineal-thyroid axes (Patel et al.,1985; Ramachandran and Patel,1986). As a corollary to the above observations, the influence of exogenous M on reproductive functions needs evaluation. Apart from M, other methoxyindoles like methoxytrytophol (ML) and methoxytryptamine (MT) have also been of late accredited reproductive functions (Reiter,1981; Pevet,1983,1985). The influence of pineal indoles in general and, their time specific effects on reproductive functions have been evaluated to a greater extent in seasonally breeding mammals in particular and, sub-mammalian species at

random (Misra and Thapliyal,1979; John et al.,1980; NG T.B.,1987; Pevet et al.,1987; Haldar and Ghosh,1988; Haldar and Pandey,1989). However, comparative effects of methoxyindoles as well as parachlorophenylalanine (pCPA), the specific depletor of serotonin leading to chemical pinealectomy, on gonadal functioning in the avian fauna have not been studied. It is in this context that the effects of M, ML, MT and pCPA on the weight, structure and functions of testis, adrenal and thyroid have been studied in the male pigeons.

MATERIALS AND METHODS :

Procurement and maintenance of pigeons :

Adult feral blue rock pigeons, Columba livia in the weight range of 250-300g, procured form a local animal dealer were used for the present study. The birds were housed in a well ventilated aviary with food and water ad libitum. After a week of acclimation, the birds were sexed using a laproscope and only males with similar testicular condition were used for the experiments.

Experimental set-ups :

In the recrudescent phase (Jan. - Feb.), a total of 36 male

pigeons were randomly divided into six groups. Two female birds were kept per group.

Group I (Control:C) These birds were given daily injections of 0.9% saline with a few drops of ethanol at 17.00h.

Group II (Melatonin morning:MM) These birds were given daily injections of 50µg melatonin at 09.00h.

Group III (Melatonin evening:ME) These birds were given daily injections of 50µg melatonin at 17.00h.

Group IV (Methoxytryptophol:ML) These birds were given daily injections of 25µg ML at 17.00h.

Group V (Methoxytryptamine:MT) These birds were given daily injections of 25µg MT at 17.00h.

Group VI (Parachlorophenylalanine:pCPA) These birds were given daily injections of 1mg pCPA at 17.00h.

All the treatments were given intraperitoneally (ip) and on the 16th day they were weighed and sacrificed by decapitation under mild ether anaesthesia taking maximum care to avoid any stress during handling.

Preparation of Solutions :

Pineal indoles (M, ML, MT) and pCPA were procured from Sigma chemicals company, St. Louis, USA. Required quantity of each

of the pineal indoles was first dissolved in a few drops of ethanol and then diluted to the required concentration with 0.9% saline. Parachlorophenylalanine was dissolved in 0.9% NaCl and brought to pH 6.0 by addition of 5 mol/l Na_2HPO_4 and stored in a refrigerator for daily use.

Parameters and methodology of evaluation :

Gravimetry : Immediately after decapitation , the viscera was cut open and testes, adrenals and thyroid were quickly excised, blotted free of blood and tissue fluids and weighed on a digital mettler balance. The absolute weights thus obtained were converted to relative weights and expressed as percentage of body weight. (Table - 1.1 ; Fig 1 A)

Histology : Tissue to be processed for histological studies was fixed (immediately after decapitation) in Bouin's fluid and processed routinely . Paraffin sections of 5μ thickness were cut on a microtome and stained with haematoxylin-eosin. The stained sections were mounted in D.P.X.

Collection of serum : Prior to decapitation, blood was collected from jugular vein and centrifuged at 4000 r.p.m. Serum thus obtained was stored at -20° C till further assay.

Serum T3 and T4 levels : Circulating levels of T3 and T4 were assayed using RIA kit provided by radiopharmaceutical division, Bhabha Atomic Research Centre, Bombay. The levels of T3 and T4 were expressed as ng/ml.

Statistical analysis : In the present study, statistical significance for all the quantitative data was determined using Student's t-test, at $P < 0.05$.

OBSERVATIONS :

Gravimeteric changes :

In general, treatment with methoxyindoles and pCPA reduced the weight of testes significantly. At an average, the reduction in weight ranged between 80% and 90%. The weight of the adrenal was significantly increased and the increase ranged from 15% to 35%. Though the weight of the thyroid increased, it was statistically insignificant. (Table-1.1, Fig - 1A).

Histological changes :

Testis : The testis of control birds in the recrudescent phase was marked by enlarging tubules with many layers of germ cells. Spermatogenesis was initiated in most of the tubules

Treatments	Relative weight (mg/100g) Testes	Adrenals	Thyroid
C	200.86 ± 6.28	6.50 ± 1.04	5.70 ± 1.80
MM	59.56* ± 10.08	8.19* ± 1.14	4.80* ± 1.24
ME	56.31* ± 6.37	7.31 ± 9.05	5.52 ± 1.11
ML	19.21* ± 4.43	9.21* ± 1.08	5.36 ± 1.37
MT	22.47* ± 7.82	8.16* ± 1.17	6.37* ± 0.91
pCPA	42.59* ± 4.14	8.80* ± 1.19	5.95 ± 1.22

Table 1.1 : Changes in relative weights of testes, adrenals and thyroid in pigeons treated with methoxyindoles and pCPA during the recrudescent phase.
(* = Significant at $P < 0.05$; values are $\bar{x} \pm SD$)

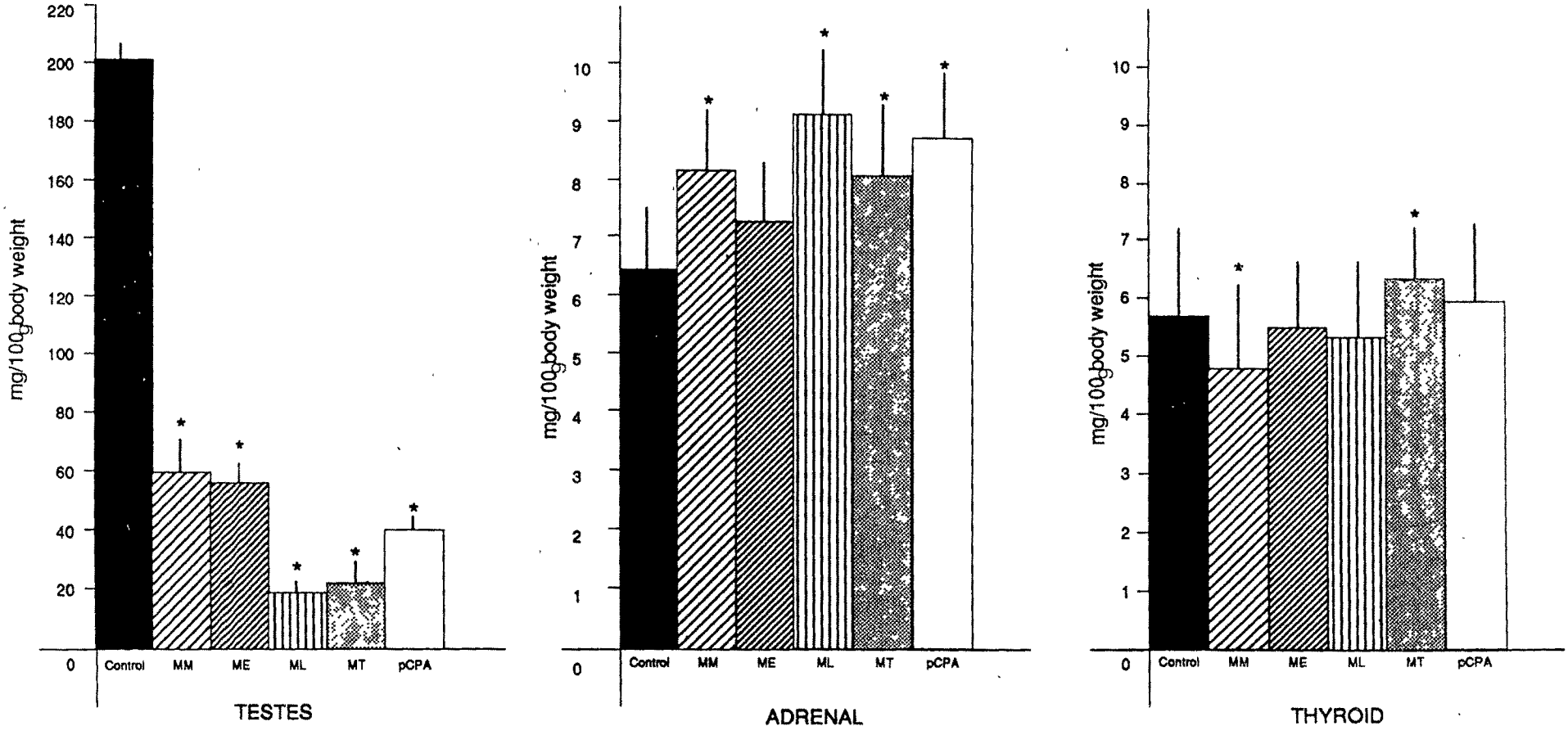


Fig. : 1.A Changes in relative weight of testes, adrenals and thyroid in pigeons treated with methoxyindoles and pCPA during the recrudescent phase
(* = Significant at $P < .05$; values are $\bar{X} \pm SD$)

and advanced stages like spermatocytes and round spermatids had made their appearance. The interstitium showed regional difference with hypertrophied cells being present in the neighbourhood of tubules containing early stages of germ cells and small reduced cells in the neighbourhood of tubules containing mature stages of germ cells.

In general, all treatments induced regressive changes. Both MM and ME arrested spermatogenesis and the diameter of the tubules was reduced. The regressive changes were more pronounced in ME in relation to MM with prominent intertubular spaces. There were hardly one or two layers of germ cells most of which were hypertrophied and exhibited nuclear pyknosis. The interstitial cells were mostly small and regressed with fibroblast like appearance. Both ML and MT also induced spermatogenic arrest and tubular regression. Relatively, the regressive effects on tubules were prominent in ML. Most of the germ cells showed degenerative changes with nuclear pyknosis. The germ cells were hypertrophied in MT treated birds in relation to ML. The interstitial cells were active and well formed in some parts and small and regressed in other parts. Treatment with pCPA also induced tubular regression and spermatogenic arrest. The basement membrane was thickened and the hypertrophied germ cells showed rampant nuclear pyknosis. The interstitium showed a mixture of hypertrophied and regressed cells.(Plates-I - III)

PLATE I

Figs 1-5 : Photomicrographs of testis of control and MM, ME,MT and pCPA treated pigeons in the recrudescent phase. (200 X)

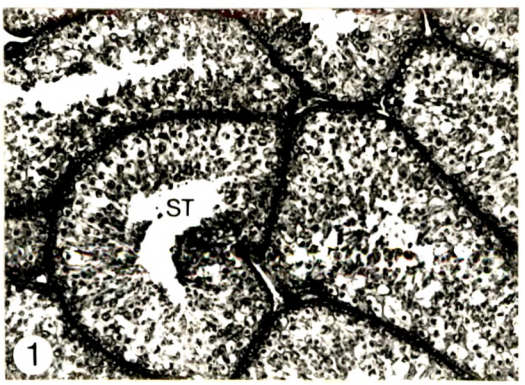
Fig 1. : Testis of control bird showing active seminiferous tubules (ST) with very little intertubular spaces.

Fig 2 : Testis of birds treated with melatonin in the morning showing reduced size of seminiferous tubules and disrupted spermatogenesis.

Fig 3. : Testis of pigeon treated with melatonin in the evening showing greatly regressed tubules containing degenerating germ cells.

Fig 4. : Testis of MT treated pigeon depicting very much regressed tubules containing only basal layer of germ cells.

Fig 5. : Testis of pCPA treated bird depicting fully regressed tubules containing degenerating germ cells.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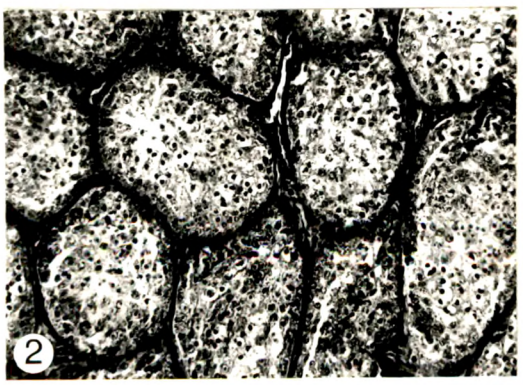
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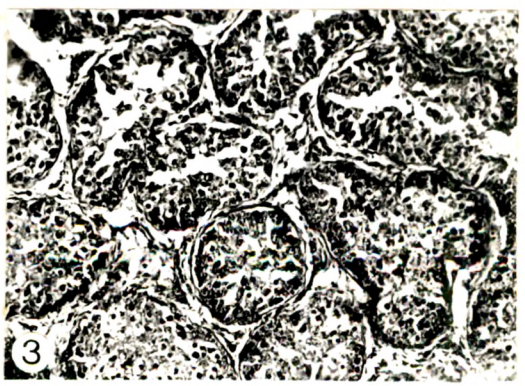
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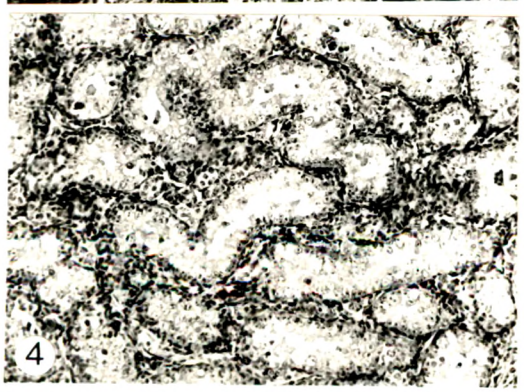
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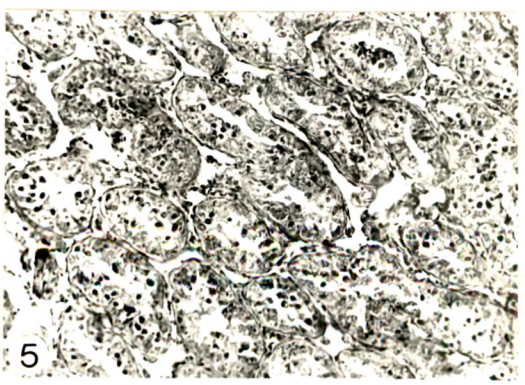
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PLATE II

Figs 6-10: Photomicrographs of testis of control and MM,ME, MT and pCPA treated pigeons in the recrudescent phase. (400 X)

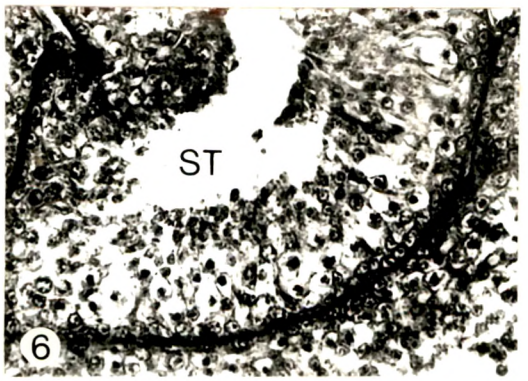
Fig 6. : Part of a tubule of control bird showing spermatogenic stages.

Fig 7. : Testis of Melatonin (morning) treated bird showing one single tubule filled with degenerating germ cells.

Fig 8. : Testing of Melatonin (evening) treated birds showing shruken tubules with degenerated germ cells.

Fig 9. : Testis of methoxytryptamine (MT) treated bird showing many highly regressed tubules with only basal layer of germ cells and prominent interstitium (IC).

Fig 10. : Testis of bird treated with pCPA depicting regressed tubules with degenerating germ cells.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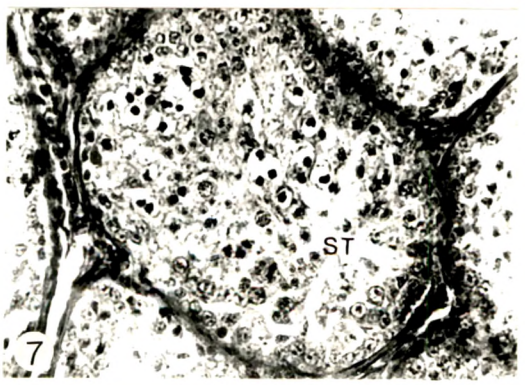
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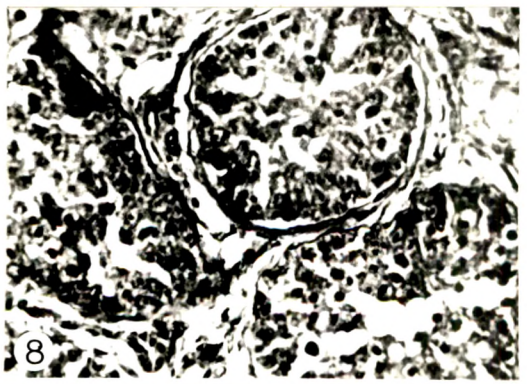
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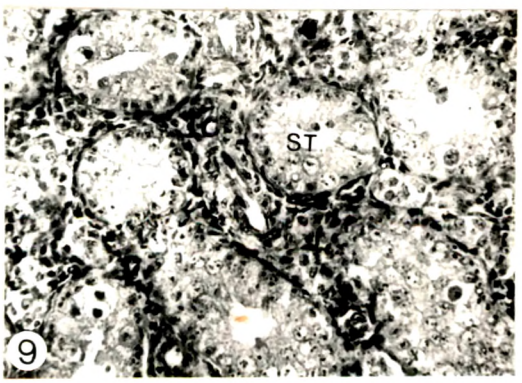
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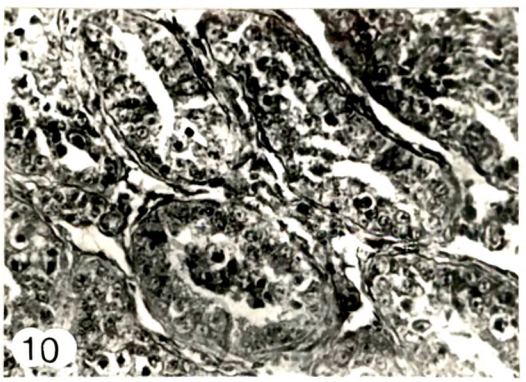
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PLATE III

Figs 11-15: Photomicrographs of control and MM,ME,MT and pCPA treated pigeons during recrudescent phase. (640 X)

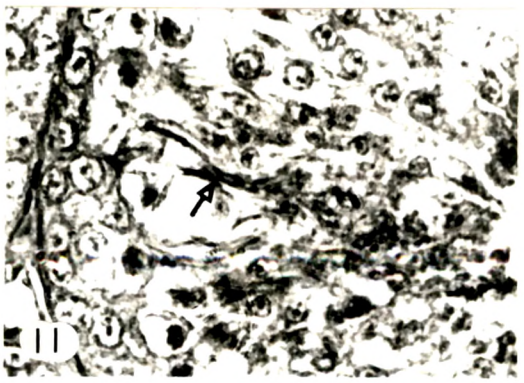
Fig 11. : Part of the tubule of control testis showing various spermatogenic stages. Note the presence of elongated spermatids (arrow).

Fig 12. : Part of a tubule of MM treated bird showing hypertrophied, degenerating germ cells with pyknotic muclei (arrow).

Fig 13. : Part of tubules of ME treated bird showing degenerated cellular debris.

Fig 14. : Tubules of MT treated bird showing only a basal layer of germ cells. Note the prominent interstitium. (IC).

Fig 15. : Single tubule of pCPA treated bird showing degenerating germ cells with pyknotic nuclei.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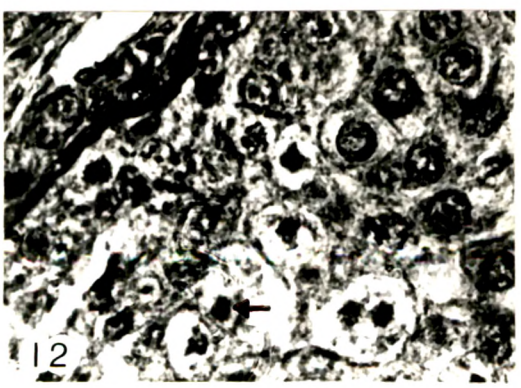
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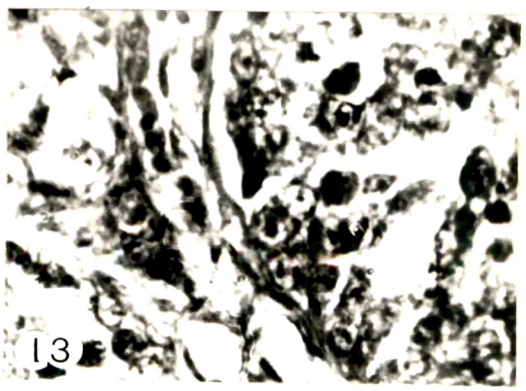
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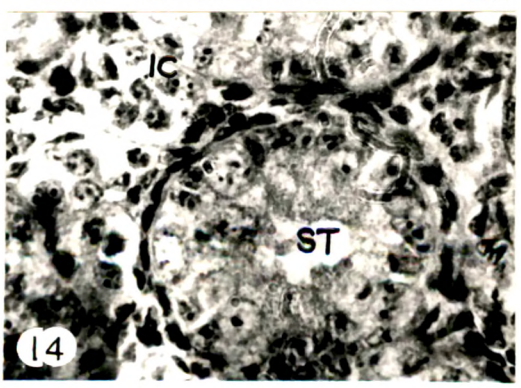
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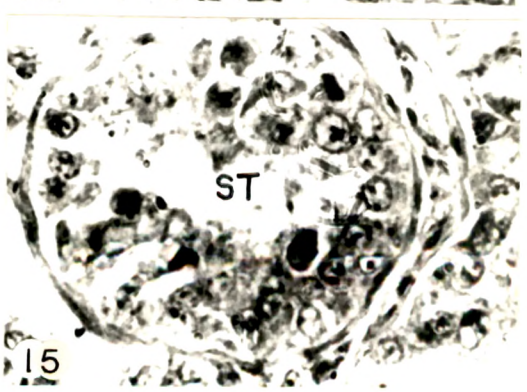
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PLATE IV

Figs 16-18: Photomicrographs of adrenal of control and melatonin and pCPA treated pigeons in the recrudescent phase. (200 X)

Fig 16. : Adrenal of control bird showing cortical and medullary areas.

Fig 17. : Adrenal of melatonin treated bird showing cortical enlargment and activation. Note the increased nuclear size.

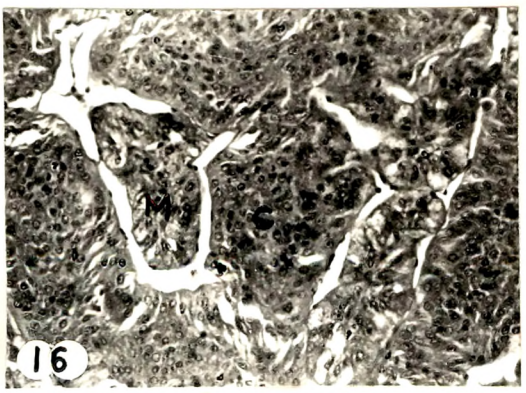
Fig 18. : Adrenal of pCPA treated bird showing cortical regression and medullary hypertrophy.

Figs 19-21: Photomicrographs of thyroid of control and M and pCPA treated pigeons (400 X).

Fig 19. : Thyroid of control bird showing follicles containing moderate amount of colloid.

Fig 20. : Thyroid of M treated bird : Note the full colloid content in all the follicles.

Fig 21. : Thyroid of pCPA treated bird showing follicles with depleted colloid content.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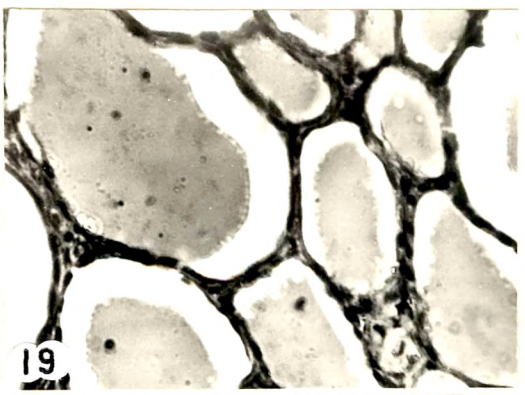
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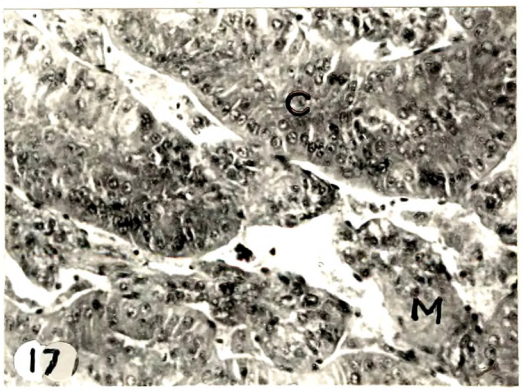
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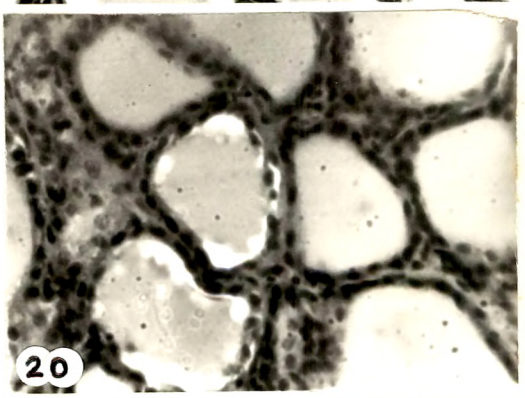
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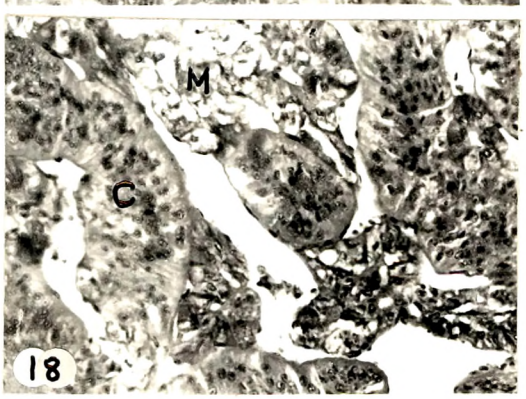
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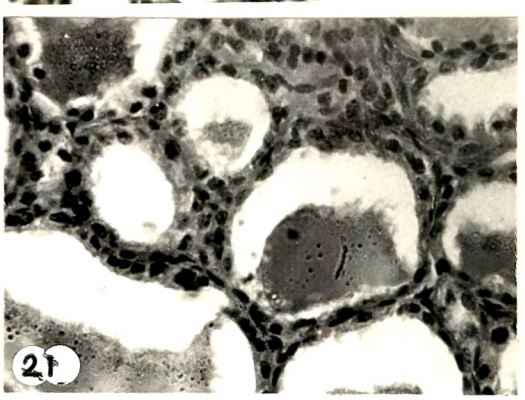
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Adrenal : The adrenal of control birds were marked by well formed cortical cords with prominent medulla scattered inbetween. Melatonin treatment (MM & ME) induced cortical enlargement with medullary regression. However, ML, MT and pCPA treatments induced medullary hypertrophy and secretory exhaustion without having much influence on the cortical structure.(Plate IV)

Thyroid : The thyroid of control birds showed medium to large follicles lined by a flattened epithelium. Colloidal content of the follicles varied depicting a mixture of fully filled, half filled or empty follicles. Treatment with MM, ME and ML brought about colloid retention in the follicles as most of the follicles were filled with colloid with no apparent change in the follicular epithelium. On the other hand, treatment with MT and pCPA induced epithelial cell hypertrophy and colloid depletion as most of the follicles were devoid of colloid. Treatment with MT also induced some degree of epithelial cell hyperplasia.(Plate IV)

Serum T4, T3 Levels :

The control pigeons showed 15.12 $\pm$ 1.13 ng/ml T4 and 3.46 $\pm$ 0.31 ng/ml T3 in the recrudescent period. Birds treated with MT and pCPA showed a significant increase in

Treatments	Serum Hormone(ng/ml) T4	T3
C	17.12 ± 2.26	3.46 ± 0.91
MM	12.22* ± 1.81	2.89 ± 0.70
ME	11.00* ± 1.28	2.50 ± 0.31
ML	10.86* ± 1.33	2.01 ± 0.20
MT	21.98* ± 2.13	3.80 ± 0.29
pCPA	23.64* ± 2.43	4.44 ± 0.24

Table 1.2 : Changes in relative weights of serum levels of T4 and T3 in pigeons treated with methoxyindoles and pCPA during the recrudescent phase.
(* = Significant at $P < 0.05$; values are $\bar{x} \pm SD$)

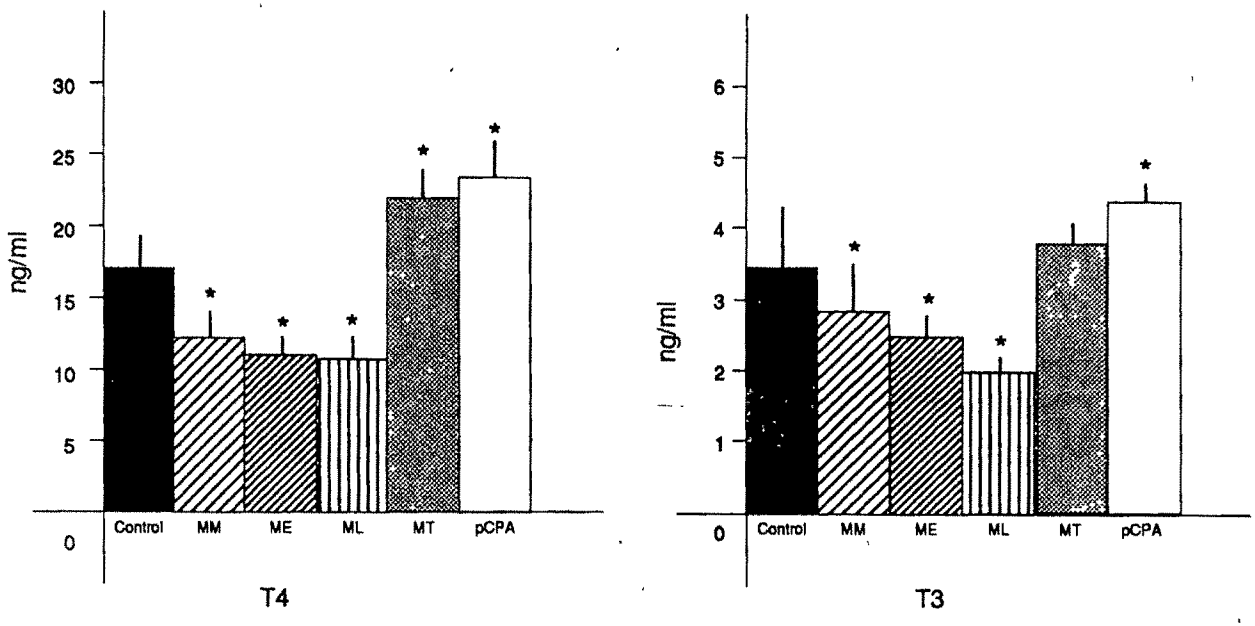

Fig. : 1.B Changes in Serum levels of T4 and T3 inpigeons treated with methoxyindoles and pCPA during the recrudescent phase.
(* = Significant at P < .05 ; values are $\bar{x} \pm SD$)

both T4 and T3 levels. Concurrently, MM, ME and ML treated pigeons showed a significant decrease in T4 and T3 levels (Table - 1.2; Fig-1B).

DISCUSSION :

The antigonadotrophic effects of M is well established in mammals (see introduction). However, the influence of M on the neuroendocrine reproductive axis of birds is not yet well defined (Gupta et al.,1987). But, circannual variations in pineal activity has been inversely related to the functional status of gonads in a tropical wild bird (Choudhuri and Maiti,1989). Further, M administration has also been reported to inhibit the normal seasonal growth of testes in another Indian bird, Lal munia, Estrilda amandava (Gupta et al.,1987).

The present study on wild pigeon has recorded involutionary changes and retardation of gonadal growth by M administration either in the morning or in the evening. In this respect, the effect of M on the reproductive axis is time independent unlike in mammals. A similar time independent action of M has been shown in the Lal munia also (Gupta et al.,1987). Histological observations show complete arrest of spermatogenesis and germ cell degeneration in the regressed seminiferous tubules of M treated birds. Even the

interstitial cells depicted an inactive state. These changes are suggestive of the inhibitory effects of M on the hypothalamo-hypophysial axis regulating testicular functions. In fact, a direct action of M on the hypothalamic GnRH neurons has been shown in the Japanese quail (Ohta et al.,1989). Interestingly, similar histological alterations in the testis of golden hamster under M treatment has been shown by Ool and NG (1989). Apart from the purported antigonadotrophic action of M, a direct action of the indole on testes also cannot be discounted as M has been shown to decrease PRL receptors and increase LH receptors in the syrian hamster (Amador et al.,1988)

The other two methoxyindoles used i.e. ML and MT also brought about testicular involution and spermatogenic arrest. This suggests that a common anti-gonadal effect of all pineal indoles in the pigeon. The convergent effect of all the three pineal indoles appears a bit intriguing when viewed in the context that ML has a different circadian rhythmicity (high during photophase and low during scotophase) in relation to M and MT, which show high levels during scotophase and low levels during photophase (Reiter,1981,1984; Skene et al.,1986,1991). But the present observation of continuous sensitivity of the hypothalamo-hypophysial-gonadal axis to pineal indoles on the diurnal time scale and also supported by the similar

observation in Lal munia (Gupta et. al.,1987) easily dispel this paradox. In this respect, the avian species seem to differ from the mammals where the sensitivity of the M changes over the diurnal time scale due to down regulation of hypothalamo-hypophysial-gonadal axis to M receptors (Reiter,1980). The time independent suppressive influence of pineal indoles on testicular functions during the breeding phase is further corroborated by the identical observation made by Haldar and Ghosh (1988) in another bird, Perdicula asiatica.

Similar to the effects of pineal indoles, chemical pinealectomy by pCPA treatment also induced testicular regression and spermatogenic arrest. This is in complete agreement with the previous findings based on surgical PX from this laboratory suggesting a progonadal role for the pineal in the reproductively active phases of pigeons (Ramachandran and Patel,1986; Ramachandran et. al.,1987). Further support comes from the inhibitory effects of PX on testicular growth in Perdicula asiatica. Apparently, the anti-gonadal effects of pineal indoles as well as PX ipso facto suggests differential action on the reproductive axis.

Previous observation from this laboratory had shown parallel pineal-adrenal and inverse pineal-thyroid axes during the breeding season in pigeons (Patel et. al.,1985; Ramachandran and Patel,1986). The present study has helped confirm these

relationships as exogenous M induced adrenocortical activation and inhibition of thyroid indicated by colloid retention within the follicles. The effect of ML on the thyroid was identical to that of M suggesting a similarity of action. On the other hand, MT and pCPA treatments increased the thyroid activity as marked by the depleted colloid content of the follicles and increased epithelial cell height. This effect of pCPA was similar to surgical PX (Patel et al.,1985; Ramachandran and Patel,1986). Validity to the inferences drawn from the histological observations are confirmed by the recorded RIA levels of T4 and T3. Support to the present findings comes form the reported decreased thyroid activity in the Lal munia (see Thapliyal, 1993) and fall in plasma T4 level in the pigeon (John et al.,1990) in response to M.

The antigonadal influence of MT and pCPA in the wake of unaltered adreno-cortical functions seems to be mainly due to the increased thyroid activity and elevated thyroid hormone levels. This is reflected in the less active state of the thyroid in the breeding season and hyperactive state in the non-breeding season in intact pigeons and the active state in PX birds during the breeding season (Patel et al.,1985; Ramachandran and Patel,1986). Moreover, the ongoing studies in this laboratory provide compelling evidence for an inverse thyroid-testes axis as indicated by

the induction of testicular regression in the breeding season by T4 administration. Many previous studies provide impressive evidence for antigonadal role of thyroid in many birds (see Pathak and Chandola,1983). Based on the studies of thyroidectomy (Chandola and Thapliyal,1978), T4 administration (Chandola and Bhatt,1982; Chandola et al.,1982) and on the circannual variations in T4 and T3 levels in the spotted munia, Lonchura punctulata, Pathak and Chandola (1983) concluded that T4 is the active principle associated with reproduction and that it has inverse relationship with gonadal activity. The present findings in the wake of the above cited reports strongly suggest that increased thyroid activity/ T4 level is the causative factor for testicular regression in the pigeon and that PX or MT treatment induces hyperactivity of the thyroid. Obviously, PX and MT seem to exert a positive influence on the hypothalamo-hypophysial-thyroid axis and the resultant surge of T4 can be surmised to either supress the hypothalamo-hypophysial-gonadal axis and/or antagonize testosterone action as evidences for both are available (see Murton and Westwood,1977; Jallageas et al.,1978).

In contrast, the testicular regression induced by M and ML seems to involve an entirely different mechanism of action as both the indoles reduced the thyroid activity. Presumably, M and ML may have a direct suppressive action on

the hypothalamo-hypophysial-gonadal axis coupled with a probable local action involving reduced responsiveness of the testicular tissue to gonadotropins (Tamarkin et al.,1977; Amador et al.,1988 ; Ohta et al.,1989).

Overall, it can be concluded from the present observations that PX as well as pineal indoles can induce testicular regression in the breeding phase. However, the mechanism of action seems to be differential as PX and MT-induced gonadal regression seems to be by way of increased thyroid hormone secretion while the regression brought about by M and ML appears to be through their inhibitory actions on the hypothalamo-hypophysial-gonadal axis. It is presumable that pineal has definite phase relationship with thyroid and adrenal.