CHAPTER III

MELATONIN REPLACEMENT TO PINEALECTOMISED PIGEONS IN THE BREEDING SEASON IS ABLE TO RESTORE THYROID AND ADRENAL FUNCTION BUT NOT THAT OF TESTES.

Previous works have shown that chemical pinealectomy as well as treatments with parachlorophenylalanine and melatonin during the breeding season can bring about testicular regression in the Indian feral pigeon (Patel et al.,1985; Chapter I). However, the progonadal effect of PX and the antigonadal effect of M at the same time appears paradoxical. Nevertheless, in the previous study (Chapter I), the pinealectomy induced testicular regression was related with the associated hyperthyroidic effect while the melatonin induced testicular involution was related to its suppressive effect on the hypothalamo-hypophysial-gonadal (HHG)axis. The pineal has been associated with photoperiod mediated seasonal reproductive activities in mammals and birds through its humoral agent, melatonin. This indoleamine hormone has been purported to exert its action by governing neuroendocrine functions. Both in long day breeding mammals like the hamster and the white-footed mouse and short day breeders like the sheep, PX could alter the normal photoperiodic responses (Barrel and Lapwood,1979; Johnston et al.,1982; Reiter,1982; Bittman et al.,1983a). In

comparison, PX studies in birds have failed to provide any definite understanding of pineal in reproduction (Ralph,1981). Studies from this laboratory on feral pigeons have shown that PX as well as M administration in the breeding season can bring about gonadal regression (see above). A natural corollary to the PX-induced effects would be to test whether replacement with the pineal humoral agent, M can negate the PX effects. Replacement studies in ewes and hamsters have provided positive results in this respect (Tamarkin et al.,1976; Bittman et al.,1983b; Carter and Goldman,1983; Watson-Whitmyre and Stetson,1983; Bittman and Karsch,1984). Since comparable studies are not carried out in birds and as we have consistently found PX-induced testicular regression in feral pigeons, the present study was designed to see whether replacement with exogenous M at 17.00 h. in PX birds simulating a normal endogenous dark time increase in M could nullify the PX effects on testes, adrenal, and thyriod.

MATERIALS AND METHODS :

Procurement and maintenance of pigeons and preparation of melatonin (M) are as outlined in chapter I.

Experimental set-ups :

In the breeding phase (March-May) a total of 36 male pigeons were randomly divided into six groups. Two female birds were kept per group.

Group I (Control:C) Intact birds were given daily injections of 0.9% saline with few drops of ethanol (vehicle).

Group II (Pinealectomy/ised: PX) These birds were subjected to pinealectomy as per the method devoloped in this laboratory (Ramachandran et al.,1984) and served as pinealectomised controls.

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

Group IV (PXV) Pinealectomised birds were given daily injections of the vehicle.

Group V (PXM50) Pinealectomised birds were given daily injections of 50µg melatonin.

Group VI (PXM100) Pinealectomised birds were given daily injections of 100µg melatonin.

All the above injections were given intraperitoneally (ip) at 17.00 h for 30 days. However, none of the parameters studied presently showed any alteration between Group I & Group III and Group II & Group IV and hence only data of Group I(C) and Group II(PX) are presented.

Parameters and Methodology of evaluation : Morphometry, Histology, collection of serum and estimation of serum T3 and T4 levels are as outlined in chapter I

Estimation of Corticosterone(B) in the serum :Corticosterone was determined by the fluorimetric method of Mattingly (1962), and expressed as µg/dl.

RESULTS :

Gravimetric changes : (Table - 3.1 Fig- 3 A)

Pinealectomy decreased testicular weight significantly by more than 50%. Melatonin treatment to PX birds further reduced the testes weight, with the higher dose of M producing maximum reduction. Adrenal weight increased post-PX while M replacement had a negating influence with the higher dose being more effective. The weight of thyroid also increased post-PX. While 50µg M had no significant effect, 100µg maintained the weight closer to control.

Histological changes :

Testis : The testis of control birds showed fully active large tubules. The tubules showed all stages of spermatogenesis including spermatids and sperm bundles. The

Treatments	Relative weight (mg/100 g body wt.)		
	Testes	Adrenals	Thyroid
Control	127.59 ± 17.66	9.80 ± 1.90	5.17 ± 1.28
PX	54.41* ± 9.20	11.04* ± 1.46	7.38 ± 1.68
PxM50	19.74* ± 3.50	10.84 ± 1.58	7.07* ± 1.81
PxM100	14.90* ± 3.48	9.87* ± 1.14	6.24 ± 1.49

Table 3.1 Changes in relative weight of testes, adrenals and thyroid in PX pigeons treated with Melatonin during the breeding season.
(* - Significant at $P < 0.05$; values are $\bar{x} \pm SD$)

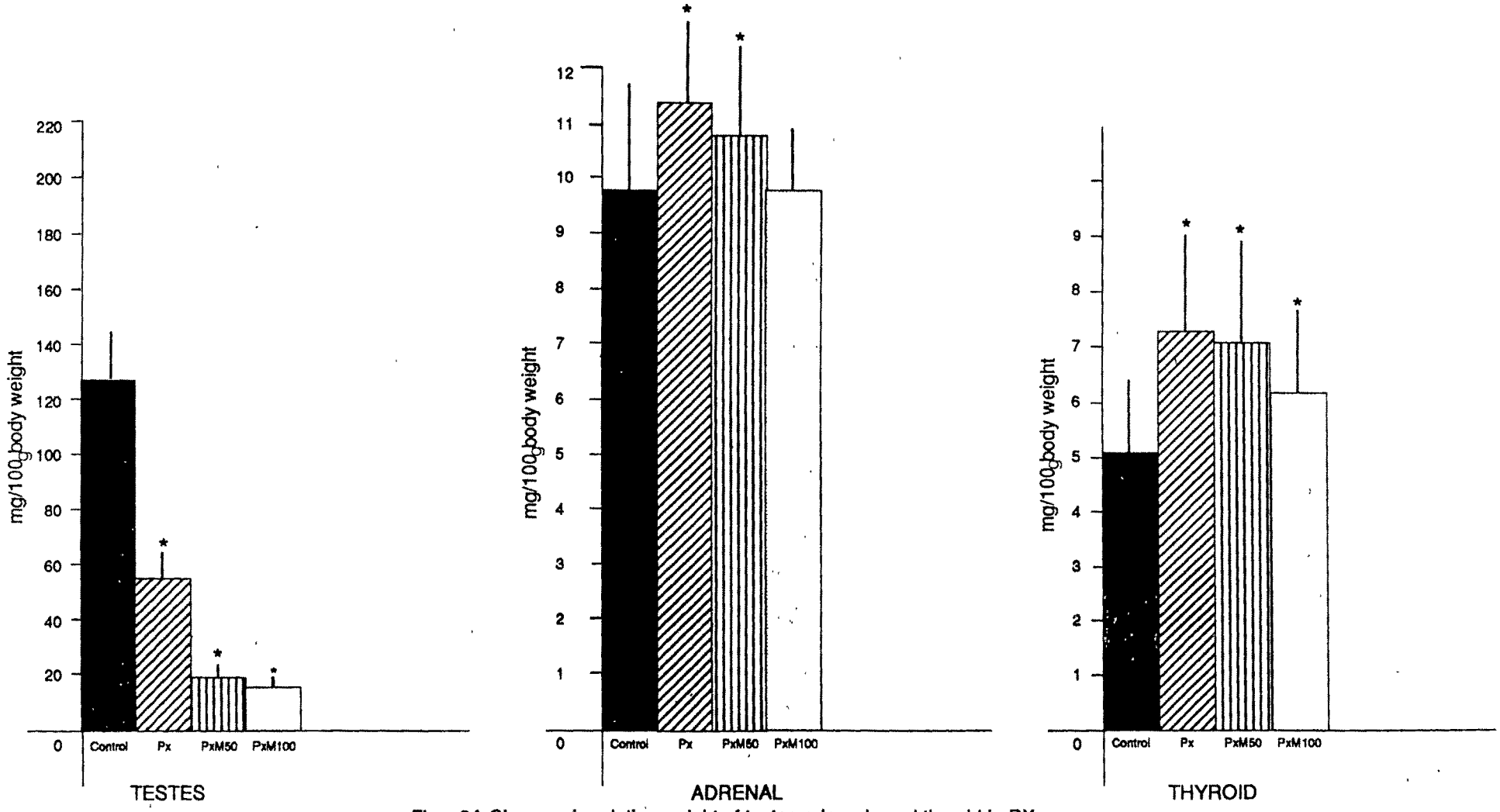


Fig. : 3A Changes in relative weight of testes adrenals and thyroid in PX pigeons treated with Melatonin (50µg or 100 µg) during the breading season. (* Significant at $P < 0.05$; values are $\bar{x} \pm$ SD).

PLATE I

Figs 1-6 : Photomicrographs of testis of control pigeons during the breeding season.

Fig 1. : Active seminiferous tubules (ST) (200 X).

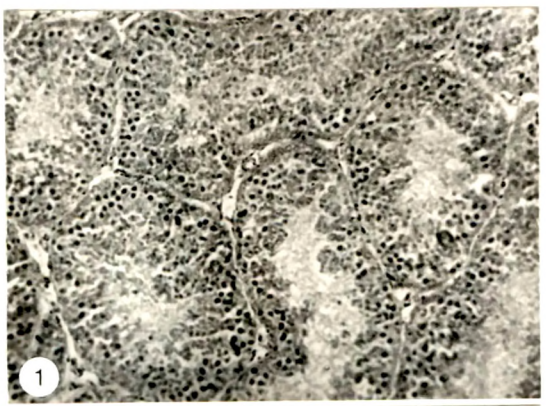
Fig 2. : One seminiferous tubule enlarged, showing active spermatogenesis (400 X).

Fig 3. : A part of a tubule showing metamorphosing spermatids. (640 X).

Fig 4. : Another region showing elongated spermatids and spermatocytes (640 X). PS = pachytene spermatocytes; EST = elongating spermatids; SS = secondary spermatocytes.

Fig 5. : Part of a tubule showing pachytene spermatocytes (PS), zygotene spermatocytes (ZS) and round spermatids (RSt).

Fig 6. : Part of a tubule showing late spermatids (LSt) bundles (640 X).



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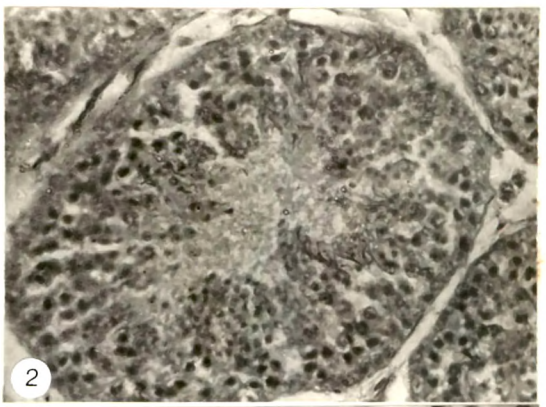

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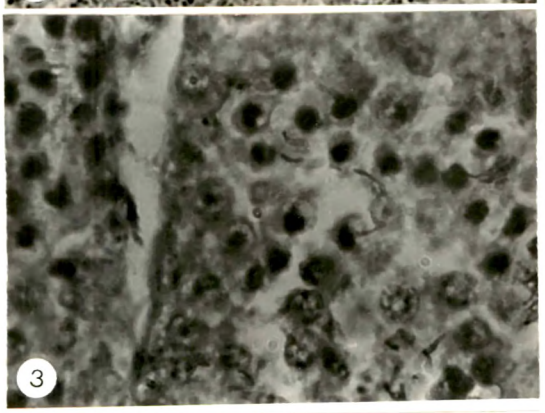

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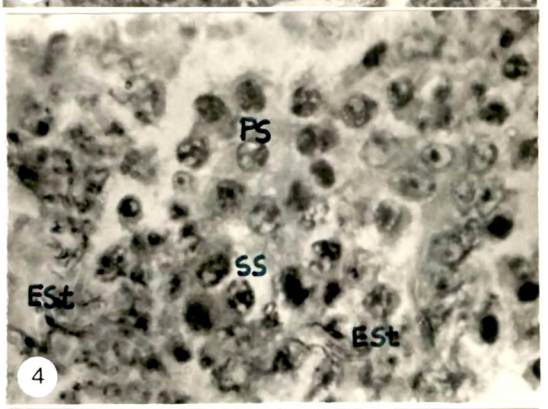

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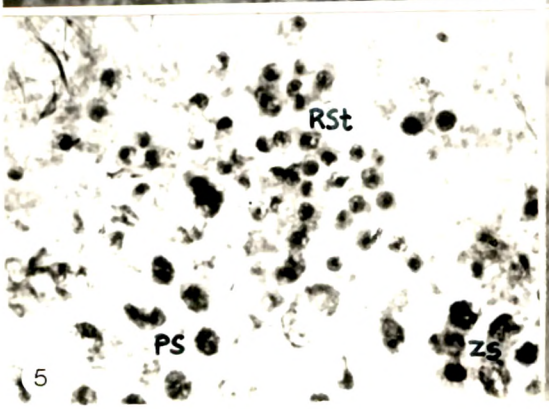

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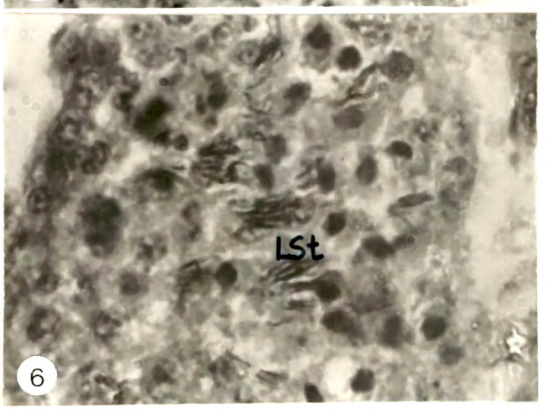

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PLATE II

Figs 7-12 : Photomicrographs of testis of pigeons subjected to pinealectomy and replacement with melatonin in the breeding season.

Fig 7. : Testis of PX pigeon showing regressed tubules with disrupted spermatogenesis, (200 X).

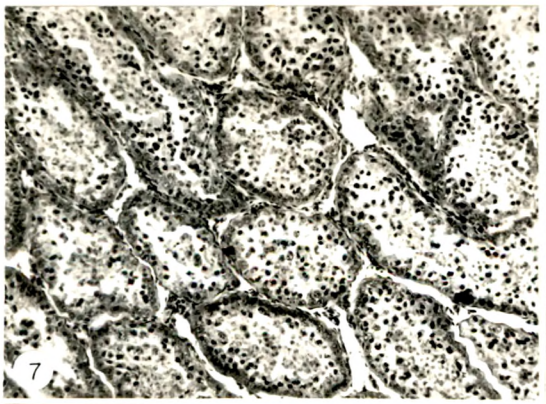
Fig 8. : Testis of PX bird replaced with melatonin, (200 X). Note the reduced size of the tubules.

Fig 9. : Enlarged version of the testis of PX bird showing arrested spermatogenesis with visible degeneration of germ cells,(400 X). Note the regressed state of interstitium also.

Fig 10. : Enlarged picture of the testis of PX bird replaced with melatonin (400 X). Note the better organization of the tubules, though spermatogenically inactive. The interstitium is well formed.

Fig 11. : A tubule of PX bird enlarged greatly to show degenerating germ cells with pyknotic nuclei. The interstitium is fibroblast like (640 X).

Fig 12. : Greater magnification of tubules of PX bird replaced with melatonin (640 X). Note the intact and active state of the basal layer of gonial cells as well as interstitial cells.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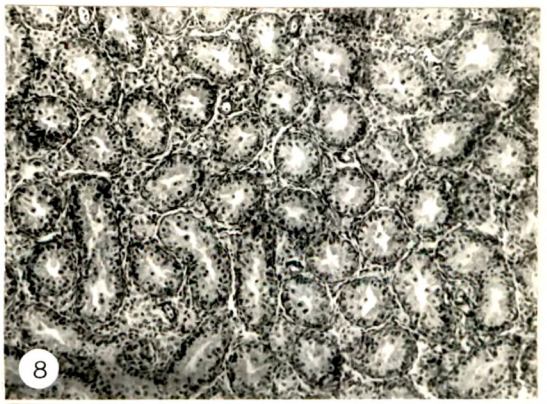
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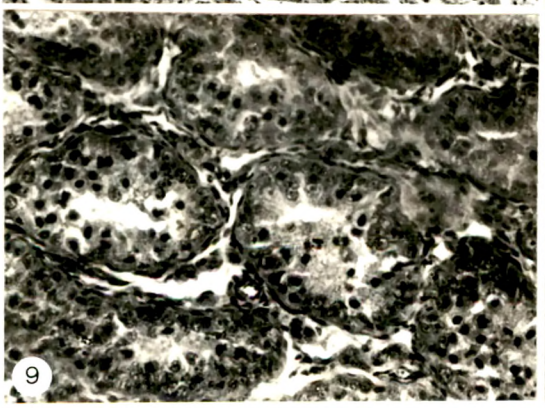
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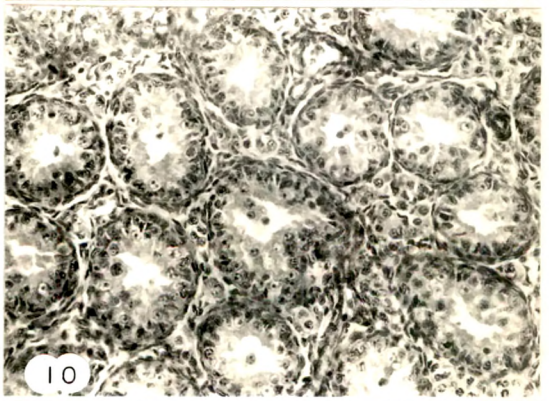
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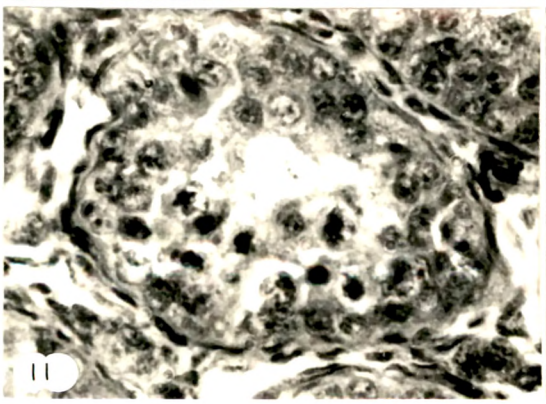
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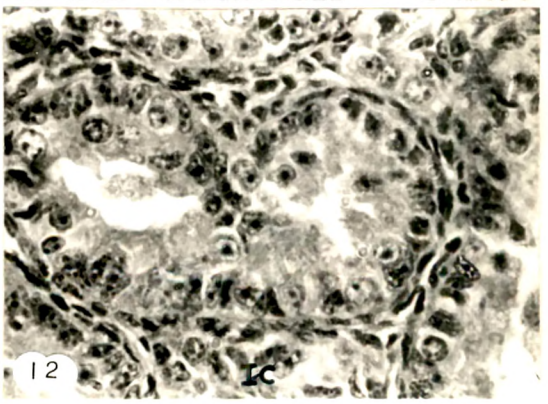
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interstitial cells were compressed in between the enlarged tubules and were mostly in fibroblastic state.Testis of PX birds showed extensive regressive changes as marked by shrunken size of the tubules showing only one or two layers of hypertrophied germ cells. Some tubules showed clumped mass of degenerating cells in the lumen. The interstitial cells were hypertrophied. Melatonin treatments showed further regressive changes. Tubules were still smaller with a single layer of hypertrophied germ cells.(Plates I,II)

Adrenal : The adrenal of control birds showed large active cortical cords with higher cortico-medullary ratio. The adrenal of PX birds showed slightly regressed cortical cords with a reduced cortico-medullary ratio. Melatonin (50µg) treated birds still showed regressed cortical condition though some hyperplastic changes were evident while birds treated with 100µg M depicted enlarged cortical cords comparable to those of the control birds.(Plate III)

Thyroid : The thyroid of control birds was marked by large turgid follicles, full of colloid and the follicular epithelium was flat. The follicular epithelium of PX birds was cuboidal and the follicles showed depletion of colloid. Thyroid of PX birds given 50µg of M was quite similar to that of PX birds though the follicles had a tendency to have more colloid. But 100µg M tended to negate the PX effects

PLATE III

Figs 13-16: Photomicrographs of adrenal of control, PX and melatonin (M) replaced pigeons in the breeding season. (200 X).

Fig 13. : Adrenal of control bird showing enlarged active cortical cords.

Fig 14. : Adrenal of PX bird showing regressed cortical cords.

Fig 15. : Adrenal of PX bird replaced with 50ug M. Note the slightly improved srtucture of the cortical tissue.

Fig 16. : Adrenal of PX bird replaced with 100ug M. Note the near normal appearance of the cortical tissue.

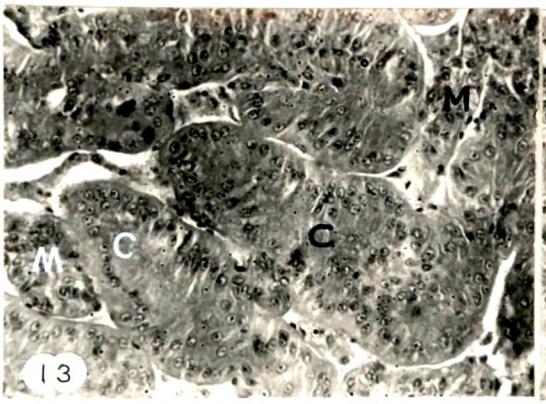
Figs 17-20: Photomicrographs of thyroid of control, PX and melatonin (M) replaced pigeons in the breeding season. (400 X)

Fig 17. : Follicles of control birds showing full colloid content.

Fig 18. : Follicles of PX birds showing depleted colloid content.

Fig 19. : Follicles of PX birds replaced with 50ug M. Note the presence of small amount of colloid in all follicles.

Fig 20. : Follicles of PX bird replaced with 100ug M. Note the near normal colloid content.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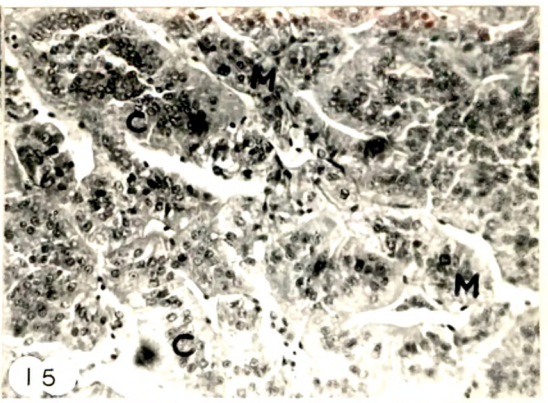
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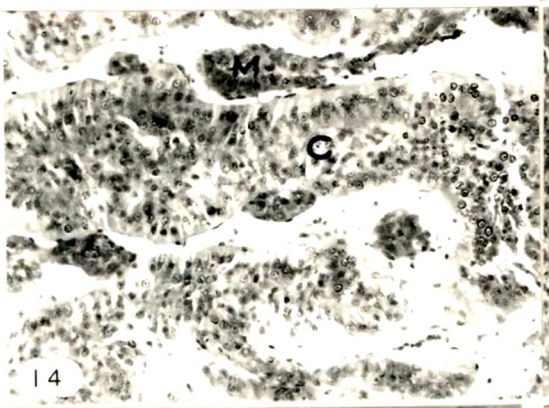
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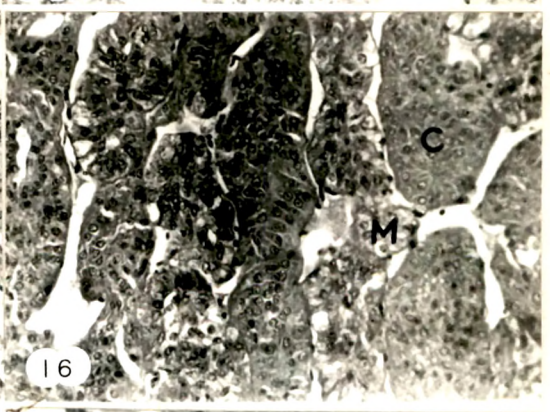
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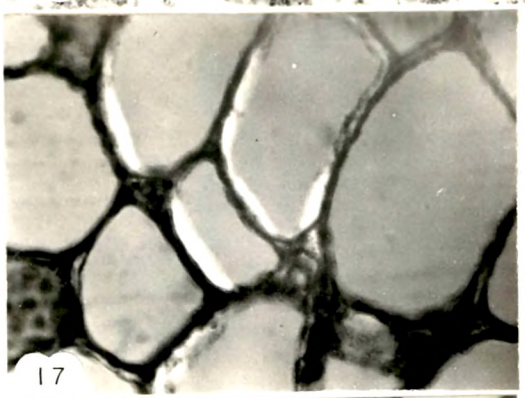
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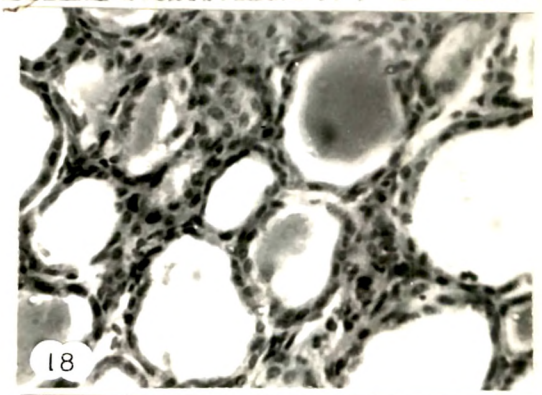
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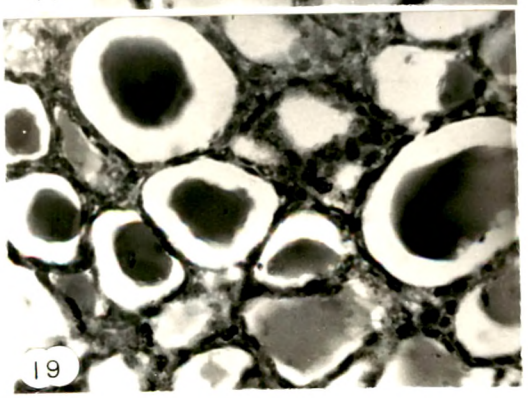
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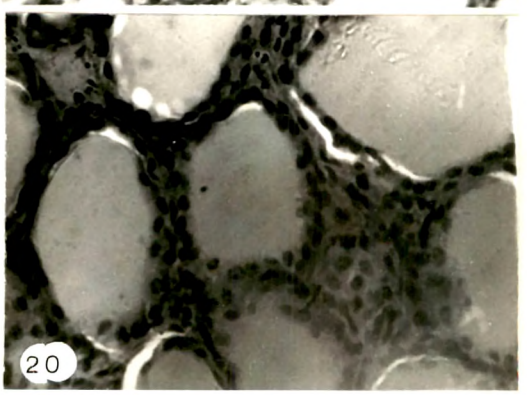
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Treatments	Serum Hormone T4 (ng/ml)	T3	B (ug/dl)
C	16.46 ± 2.14	3.01 ± 0.51	8.95 ± 1.48
Px	22.84* ± 2.81	4.08* ± 0.44	7.02* ± 1.34
PxM50	20.98* ± 4.80	3.88 ± 0.50	7.32 ± 1.46
PxM100	17.13 ± 2.17	3.33 ± 0.73	8.27 ± 2.00

Table 3.2 Changes in serum levels of T4, T3 and corticosterone (B) in PX pigeons treated with Melatonin during the breeding season.
(* = Significant at $p < 0.05$; values are $\bar{x} \pm SD$)

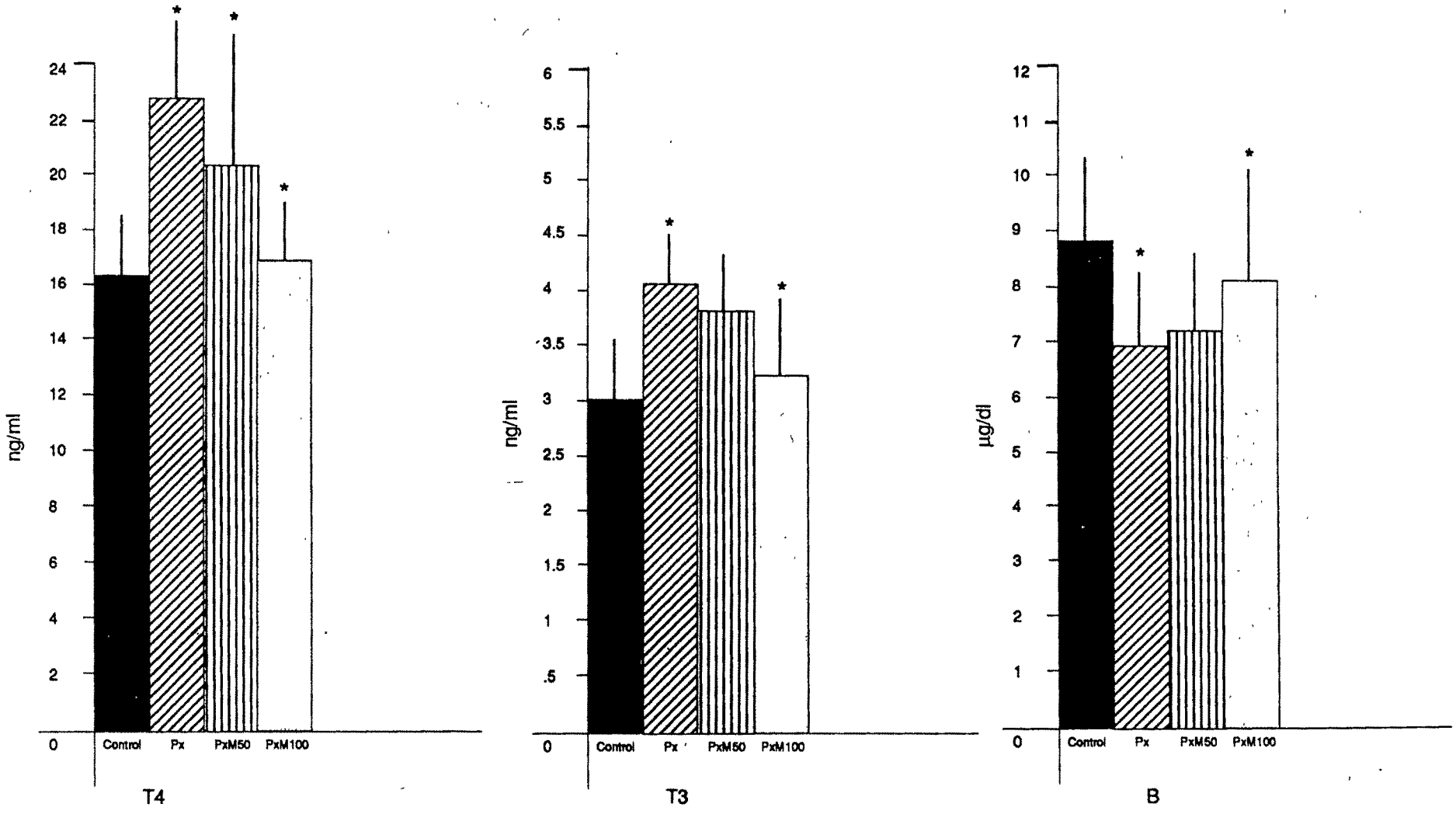

Fig. : 3B Changes in serum levels of T4, T3 and corticosterone (B) in PX pigeons treated with Melatonin during the breeding season.
(* - Significant at $p < 0.05$; Values are $\bar{x} \pm SD$).

with the result that the follicles were large and the epithelium was low cuboidal.(Plate III)

Serum T4, T3 and Cotricosterone (B) levels :

Serum T4 and T3 levels were significantly increased in PX birds. While 50µg M treated PX birds did not show much change as compared to PX birds, 100µg M treated PX birds showed T4 and T3 levels comparable to those of the control birds. In contrast, the B level was significantly decreased in PX birds. Again, 50µg M treated birds showed a level similar to that of PX birds and 100µg M treated birds showed a hormone level comparable to the control birds. (Table-3.2 fig-3B)

DISCUSSION :

The present findings provide evidence for M to be a humoral agent capable of influencing neuroendocrine axes in the feral pigeon. A suggestion that M may act within the pineal to elicit the release of another functional principle in mammals (Reiter,1974) stands discounted by the present observations. Previous studies from this laboratory have suggested phase linked interactions between hypothalamo-hypophysial- thyroid (HHT), hypothalamo- hypophysial-adrenal (HHA) and hypothalamo-hypophysial-gonadal(HHG) axes

in the control of reproductive functions in the pigeon (chapter I). The current results attest to two points, 1. Melatonin is a neuroendocrine modulator capable of manifesting its effects even in the absence of the pineal. 2. Chronic evening M injections to PX birds simulating normal nocturnal elevations in circulating M level is capable of modulating the HHT and HHA axes in the pigeon. This apparently may suggest a time dependent influence of M. Such a conclusion is however discounted by the fact that morning injections to PX birds were equally effective as evening injection (unpublished observation) as in the case of intact animals (chapter I). Studies in mammals involving M injections to intact and PX animals have also shown time/phase independent effect of M in controlling reproductive functions (Bittman et al.,1983; Carter and Goldman,1983 a,b; Bittman and Karsch,1984).

Interestingly in the present study, of the two doses of M employed, it is only the higher dose which could negate the effects of PX on the HHT and HHA axes as denoted by the histomorphological changes as well as the serum hormonal profile. Despite these effects on HHT and HHA axes, both doses of M were unable to prevent the PX-induced suppressive effects on the HHG axis. In fact, the histomorphological observations suggest potentiation of the suppressive effects on the testes. Obviously,single injection of M leading to

greater amplitude but short duration M profile seems to be insufficient to maintain the functional potency of the HHG axis though it is successful in maintaining the HHT and HHA axes in a state conducive for testicular functions. It is already reported for the pigeon, that PX does neither totally eliminate circulating M nor obliterate the circadian rhythm (Vakkuri et al.,1985). These workers reported that extrapineal sources of M is able to maintain circulating M levels to 60% of control levels after PX but,significantly there was reduced scotophase:photophase ratio as well as reduced hypothalamic M content.The latter aspect may have a significant bearing on the PX-induced inhibition of the HHG axis. On a speculative vein it can be surmised that the PX-induced reduction in M may inhibit the hypothalamic GnRH pulse generator by inhibiting serotoninergic mechanisms as, both the role of serotononergic mechanism in inducing gonadotrophin release as well as the ability of M for increased 5-HT turnover in the hypothalamus are well recorded in the literature (De Villalobos et al.,1984; Hall et al.,1986; Wainer et al.,1988; Vriend,1991).

The inability of single injection schedules leading to short duration, high amplitude M level in preventing the inhibitory effects of PX on HHG axis may suggest the need for an optimum level of M to prime the HHG axis for a certain minimum duration. Evidence for this comes from the

ongoing studies demonstrating the ability of small dose M implantation in PX birds to totally prevent the testicular regression occurring due to PX. The concept of duration effect of M in modulating the functions of HHG axis in mammals is well known and well accepted (Carter and Goldman,1983,a,b,; Bittman and Karsch,1984; Bittman et al.,1983). In the present context it is worthwhile to recall the statement of Osol et al. (1985) that "if melatonin is involved in reproductive cycles, as a considerable volume of research suggests, increasing circulating concentrations within a short time may not be critical since many animals, particularly birds, breed during only a few months of every year". Just as there is a need for an optimum-lower threshold level, of M sufficiently critical duration, to keep the HHG axis functioning in the reproductively active phase, there is also an optimum higher threshold level which could render the HHG axis non-functional as it happens during the end of a normal reproductive phase (regression) or as could be induced by either single injection or low dose implantation of M to a pineal intact animal in the breeding phase(unpublished evidence). Previous findings have shown a parallel HHG and HHA axes and an inverse HHG & HHT axes in the breeding phase of feral pigeons (chapter I) thereby sugesting an intricate phase relationship between the hormones of three axes in regulating reproductive activity. Both PX as well as M

administration to intact animals seem to alter the phase relationship thereby inducing testicular regression. Based on previous findings, PX-induced gonadal involution induced by M administration was accredited to its ability to suppress HHG axis directly though at the same time maintaining the HHT and HHA axes in the more favourable state (chapter I). The present findings are in conformity with the above and further suggest that M has independent ability of maintaining HHG axis on one hand and HHT and HHA axes on the other hand by its differential effects.