CHAPTER V

EFFECTS OF PINEALECTOMY OR EXOGENOUS MELATONIN ON SERUM HORMONE LEVELS AND HISTOMORPHOLOGY OF TESTES, ADRENAL AND THYROID OF FERAL PIGEONS IN THE SEXUALLY QUIESCENT PHASE.

Studies involving pinealectomy or melatonin (M) in birds have generated such divergent results that assigning a common function for pineal in birds has remained at best a matter of conundrum. A review of available literature in this context has compelled Ralph (1981) to opine that the functions of avian pineal remains frustratingly enigmatic due to reasons such as usage of fewer number of species and limited experimental manipulations leading to paucity of information base. Amongst mammals the role of pineal in modulating seasonal reproductive activities is well established. Accordingly, long day breeders show gonadal involution under short photoperiods or M administration and the effect of short photoperiod can be prevented by pinealectomy (Reiter,1981,1984). Conversely, in short day breeders increasing M concentration or short photoperiod induce gonadal activation which can be hindered by pinealectomy (Amador et al.,1988; Carter and Goldman,1983).

In case of birds the effects of either PX or exogenous M are not that well defined and functions ranging from progonadal

to antigonadal or both or even no effect have all been suggested (See Ralph,1981). Even the few later studies have not done much to eradicate the situation due to reports such as no effect of PX on testes function during recrudescent or active phases (Haldar and Ghosh,1988a) or early initiation of testicular development by M implantation during the testicular inactive phase (Haldar and Ghosh,1988b). Lack of sustained and detailed investigations in any individual species seems to be the reason behind the current impasse in understanding pineal functions in birds. In this context investigations initiated in this laboratory on pinealectomised feral pigeons have established parallel pineal - adrenal - gonad (PAG) and inverse pineal - thyroid -gonad (PTG) axes (Patel et al.,1985; Ramachandran et al.,1987). However, similar studies conducted on domestic pigeons revealed both the axes to be parallel (Ramachandran and Patel,1986,1988). Whereas PX in the breeding phase induced testicular involution in both, PX during the regression phase prevented the normally occuring testicular regression only in the domestic pigeons (Ramachandran et al.,1987; Ramachandran and Patel,1988). Further, previous study on feral pigeons showed proadrenal, antithyroid and antigonadal effects of exogenous M during the breeding season (Chapter I). Subsequently, it was shown that M replacement to PX birds can to a certain extent nullify the effects on the HHA and HHT axes without however altering the

HHG axis (Chapter III). Since most of the above studies was related to pineal functions in the breeding season, there was a need to evaluate the role of the pineal, if any, in the non-breeding phase. Hence in the present study, the influence of PX or exogenous M administration to intact birds has been carried out in the reproductively quiescent phase, to assess their impact on HHG, HHA and HHT axes.

Materials and Methods :

Procurement and maintenance of pigeons and preparation solution (melatonin) are as outlined in Chapter I.

Experimental set-ups :

In the quiescent phase (Aug. - Nov.) a total of 24 male pigeons were divided into four 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.

Group II (Pinealectomised : PX) These birds were subjected to pinealectomy.

Group III (Sham Pinealectomised : SPX) These birds were sham operated.

Group IV (Melatonin 50µg : M50) These birds were given daily injections of 50µg melatonin.

The injections were administered intraperitoneally (ip) at 17.00 h for 30 days. As none of the parameters studied presently showed any alteration between Group I and Group III : only data of Group I(C) are presented.

Parameters and Methodology of evaluation : as outlined in Chapter I & III.

RESULTS :

Relative weights : The control birds had reduced adrenal weight and increased thyroid weight during the non-breeding season when compared with those in the breeding season. Whereas, PX did not show much change in the weight of adrenal or thyroid, M administration decreased the weight of the latter and increased that of the former. The weight of the regressed testes showed further reduction in PX as well as M treated birds. (Table- 5.1, Fig- 5 A)

Histology : The testis of control birds in the non-breeding season showed highly regressed seminiferous tubules with large intertubular interstitial spaces. The tubules were lined with only a basal layer of gonial cells

	Relative weights (mg/100 g body wt)		
	Testes	**Adrenal**	**Thyroid**
C	38.32 ± 4.75	6.77 ± 0.83	7.42 ± 0.63
PX	23.83* ± 2.17	6.30 ± 0.31	7.57 ± 0.81
M50	14.58* ± 1.79	8.14* ± 0.94	5.77* ± 0.35

Table 5.1 : Alterations in relative weights of testes, adrenal and thyroid of PX and Melatonin treated pigeons during the quiescent phase.
(* = Significant at $P < 0.05$; values are $\bar{x} \pm SD$)

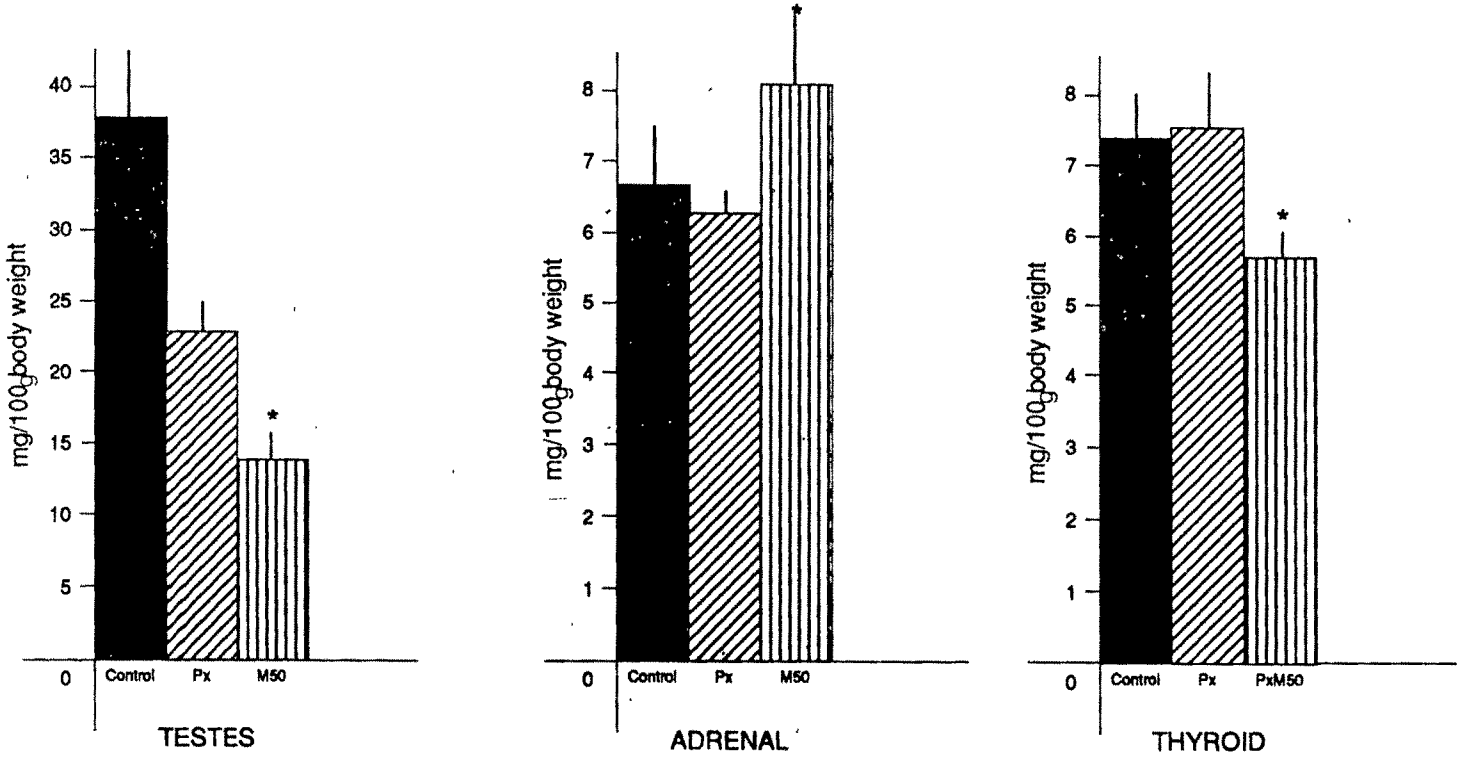

Fig. : 5A : Alterations in relative weights of testes, adrenal and thyroid of PX and melatonin treated pigeons during the quiescent phase.
(* = Significant at $P < 0.05$; values are $\bar{x} \pm SD$).

PLATE I

Figs 1-4 : Photomicrographs of testis of control and melatonin (M) treated pigeon during the quiescent phase.

Fig 1& 2 : Tubules of control pigeon showing only a single basal layer of hypertrophied gonial cells. Interstitium is fibroblast like. (200 X and 640 X respectively).

Figs 3 & 4 : Tubules of M treated birds showing the presence of more cells in the tubule and hyperplasia of peritubular cells (arrow) (200 X and 640 X respectively).

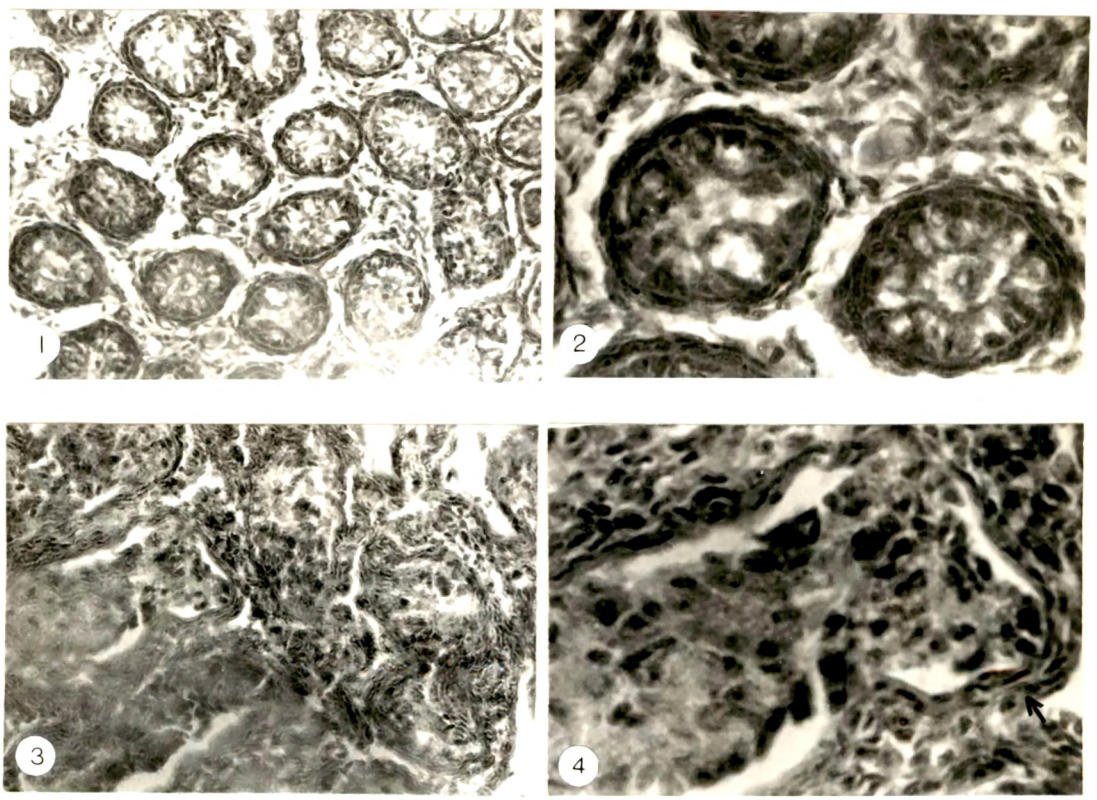
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PLATE II

Figs 5-7 : Photomicrographs of adrenal of control, PX and melatonin treated pigeons in the non.breeding season (200 X).

Fig 5. : Adrenal of control bird showing regressed inactive cortical cords.

Fig 6. : Adrenal of PX bird showing further regressed cortical tissue.

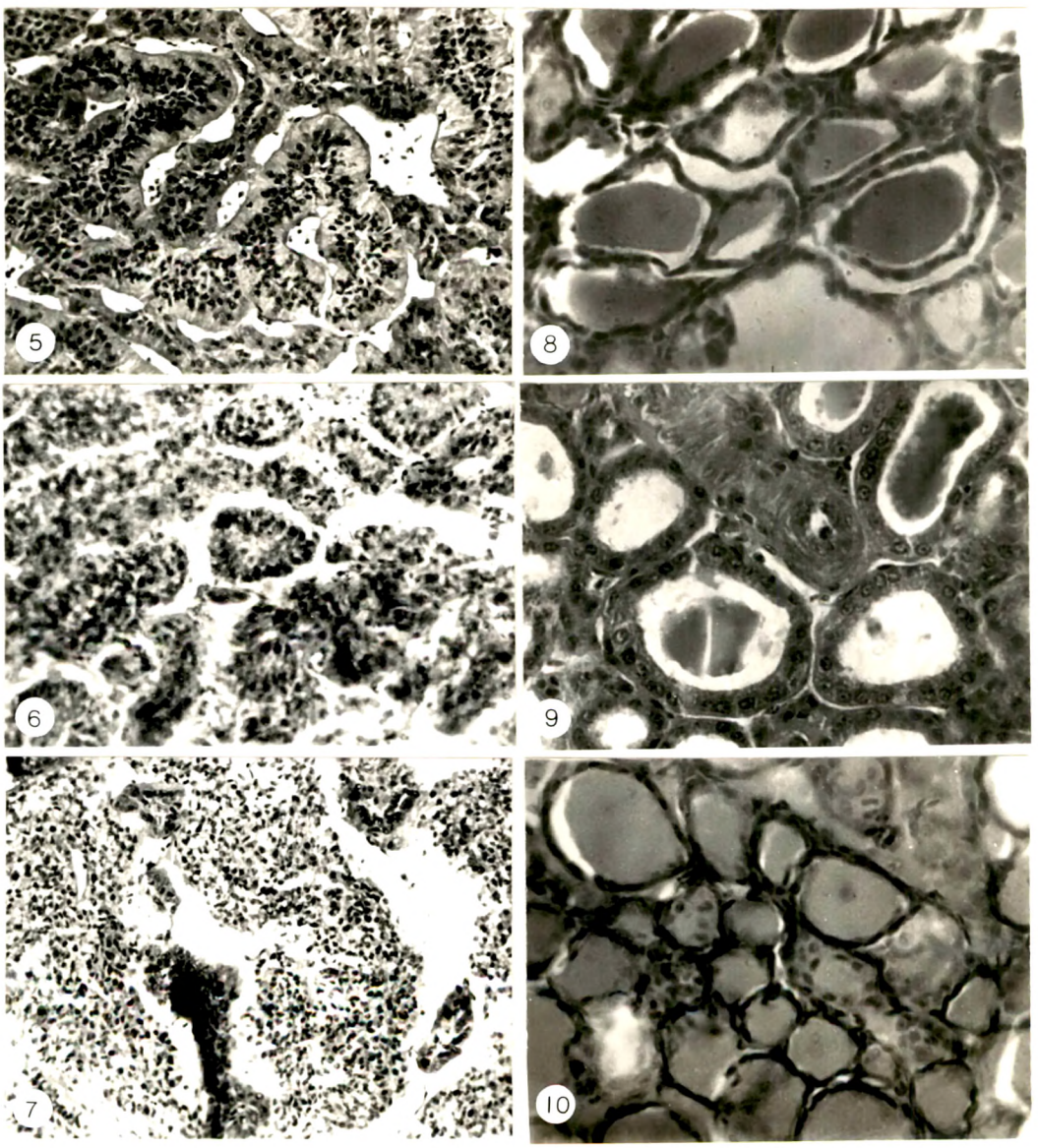
Fig 7. : Adrenal of M treated bird showing hyperplasia of cortical cells.

Figs 8-10 : Photomicrographs of thyroid of control, PX and melatonin treated pigeons in the non-breeding season. (400 X)

Fig 8. : Follicles of control bird showing varying degree of colloid content.

Fig 9. : Follicles of PX bird with depleted colloid content.

Fig 10. : Follicles of M treated bird showing full colloid content.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and the interstitial cells were highly regressed and indistinguishable from the other connective tissue cells. The tubules of both PX and M treated birds remained regressed and even showed further shrinkage. The single layer of germ cells were generally hypertrophied. In the M treated birds, the interstitial cells as well as the peritubular cells showed extensive hyperplasia. (Plate I)

The adrenals of control birds depicted cortical involution. The thyroid follicles showed varying degree of colloid depletion and were lined by low cuboidal epithelium. In general, PX birds showed more or less the same picture. However, M treatment was marked by medullary regression and extensive cortical hyperplasia in the adrenals and retention of colloid in the thyroid as marked by slightly reduced follicles filled up with colloid with slightly reduced epithelial cell height. (Plate II)

Serum T4 & T3 levels : The control pigeons had relatively higher levels of T4 & T3 in the non-breeding season. Though PX did not significantly alter the hormone levels, M treatment significantly decreased their levels. (5.2 ; Fig-5B)

Serum corticosterone (B) level : Relatively, the serum level of B was lower during the non-breeding season in the

Treatments	Serum Hormones		
	T4 (ng/ml)	T3	B (ug/dl)
C	22.64 ± 2.18	2.98 ± 0.17	7.50 ± 0.63
Px	23.21 ± 2.09	3.11 ± 0.36	7.27 ± 0.80
M50	16.77* ± 1.81	2.10* ± 0.21	7.41 ± 0.93

Table 5.2 :Alterations in serum levels of T4, T3 and corticosterone (B) of PX and Melatonin treated pigeons during the quiescent phase.
(* = Significant at $\underline{P} < 0.05$; values are $\bar{x} \pm SD$)

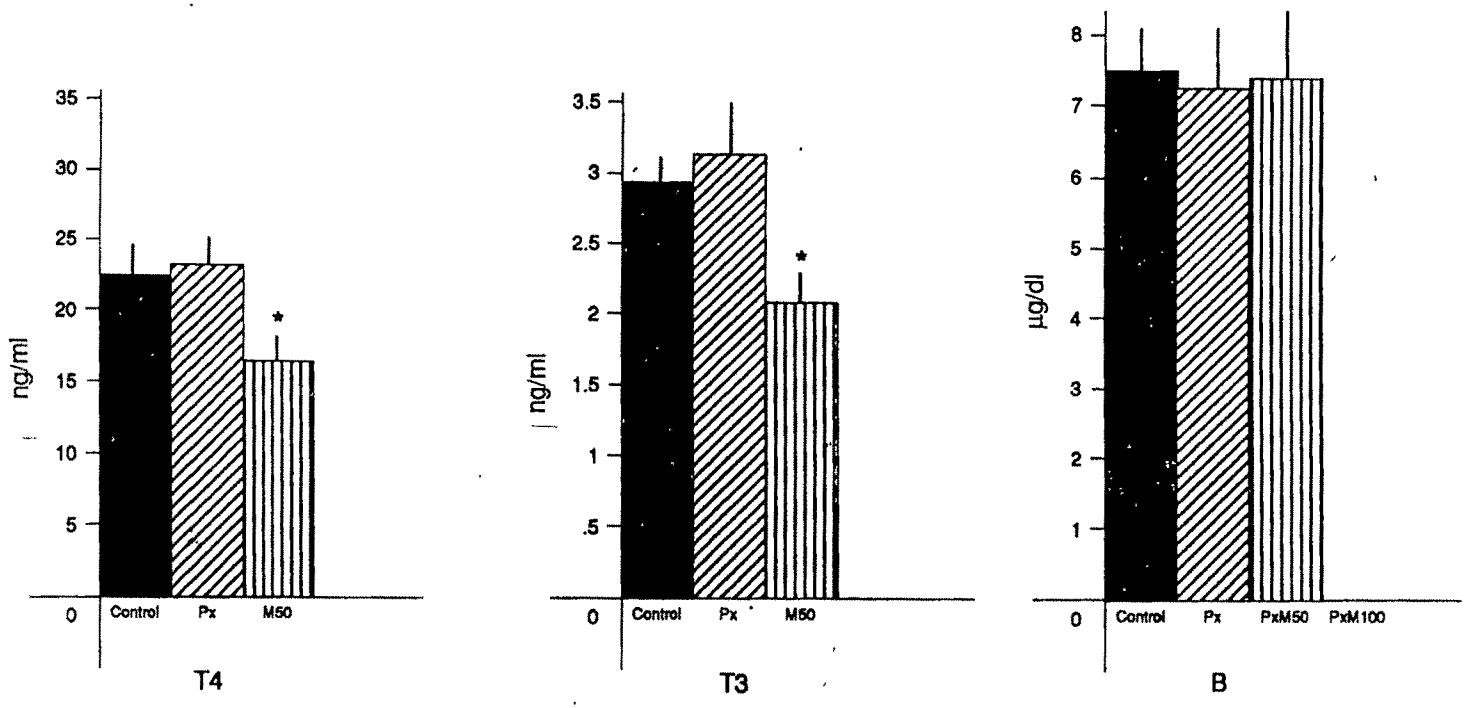

Fig. : 5B : Alterations in serum levels of T4, T3 and corticosterone (B) in PX and melatonin treated pigeons during the quiescent phase.
(* = Significant at $P < 0.05$; values are $\bar{x} \pm SD$).

control birds. Neither PX nor exogenous M had any significant effect on the serum B level.(Table-5.2; Fig-5B)

Discussion :

The current study principally undertaken to test the effect of PX or exogenous M during the testicular quiescent phase has helped confirm the fact that both sub- as well as supra-optimal levels of melatonin have a suppressive influence on the HHG axis as was inferred earlier (Chapters I & III). This is obvious by the observed reduction in the weight of testes as well as the histological changes. The previous observations of testicular involution by either PX or exogenous M in the breeding season (Ramachandran et al.,1987; Chapter I), taken together with the present observations suggests an antigonadal action of both PX and exogenous M in all seasons. In view of the nonfunctional state of the HHG axis in the non-breeding season, the presently observed decrease in the relative weight of testes as well as the histological changes in M treated birds do not discount the possibility of a direct action of this indole on the testes. It is also obvious that PX in the non-breeding season is of no consequence on the functioning of either HHT or HHA axis. This is understandable in the context that the reduced adrenocortical activity and the increased thyroid activity characteristic of the

non-breeding season are exactly reverse of the conditions in the breeding season (Patel et al.,1985) and, changes which could be induced by PX in the breeding season (Patel et. al.,1985; Chapter I). Apparently, the effects of PX on HHT and HHA axes are the same in feral pigeons on a circannual basis irrespective of either breeding or non-breeding phase. Similarly, the effect of exogenous M also seems to be the same irrespective of the season on HHT & HHA axes i.e. suppression of the former and activation of the latter. Though reduced thyroid activity coupled with increased adrenal activity are conducive for testicular functions as during the recrudescent and breeding seasons, similar changes induced by M administration during the non-breeding phase were however without any effect on the suppressed HHG axis. It appears, has also been discussed previously (Chapters I & III), that the HHG axis is refractory. Though the mechanism of this insensitivity remains yet unknown, it is very clear that reduced T4 and increased B levels are able to act on the sensitive phase of this axis to bring about testicular recrudescence and also maintain it in the active phase.

Another aspect which gets emphasised, from the present observations in the non-breeding phase, as well as from those of the past involving intact and PX pigeons during the breeding season (Chapters I & III), is that while acute elevation in M levels can suppress the HHT axis and activate the

HHA axis, an optimum level of M persisting chronically for a minimum period of time is needed to influence the HHG axis, as has been inferred by the studies involving M replacement in PX pigeons (Chapter III). Since there are no comparable studies of this nature in other avian species, it is difficult to make a generalised discussion. In an earlier study, PX-induced testicular regression in the breeding season was accredited to the suppressive action of increased T4 titre on the HHG axis (Chapter I), a mechanism which seems to be responsible for inducing testicular regression in intact normal birds. In view of these inferences, a couple of aspects that need further clarification are the possibility of refractory state of the HHG axis during the reproductively quiescent phase and the consequent mechanism involved in the termination of this refractory state to initiate a new cycle of testicular activation.