CHAPTER VII

Functional alterations of testes, adrenal and thyroid in intact and pinealectomised pigeons exposed to long photoperiod prior to recrudescent phase.

Seasonal reproduction in birds is essentially a matter of appropriate phase relationship between various endogenous neuroendocrine secretions organised by environmental factors. Photoperiod appears to be the primary environmental clue influencing the many endogenous rhythms of neuroendocrine secretion in temperate species (see Murton and Westwood, 1977; Follet and Robinson,1980; Follet, 1984; Nicholls,1988). Even the tropical birds are known to regulate their reproductive activities in relation to the change in ambient photoperiodism though, other factors like temperature and humidity have also been implicated in specific instances (see Thapliyal and Gupta 1989).The gonadal activity of a number of tropical and subtropical avian species has been shown to respond to photoperiodic manipulations (Maitra, 1986, 1987a,b).

The tropical feral pigeons have their peak breeding phase coinciding with the summer months (March-May), though some members of the population may show visible expressions of breeding behaviour during Sept-Oct., thereby giving the

impression of an extended/protracted breeding phase. The percentage of reproductive success is never-the-less very meager during such secondary phase of breeding activity. The pigeons enter into a rapid phase of gonadal regression (May-June) and remain in the quiescent state till Dec. By Jan., gonadal recrudescence commences and,full testicular size is attained by Feb. The phases of gonadal recrudescence and regression roughly correspond to the winter and summer solstice respectively. It is likely that the consequent circadian variation in M levels could modulate the neuroendocrine axis of reproduction. Similar activation of testes paralleling gradually increasing photoperiodism following winter solstice has also been reported for other tropical birds like the parakeet, Psittacula cyanocephala (Maitra,1986) and the brahminy myna, Sternus pegodarum (Kumar and Kumar, 1991, 1993). The above workers have also reported a post-breeding photorefractory phase in these birds. Exposure of brahminy myna to long daily photoperiod (LD 15:9) during the recrudescent period has been shown to stimulate full gonadal growth (Kumar and Kumar, 1991). Similarly, a nine week pretreatment with short days (LD 8:16) followed by exposure to long day lengths was found capable of dissipating photorefractoriness in these birds (Kumar and Kumar,1992). Though the possibility of photorefractoriness has been suggested in the parakeet also (Maitra,1987a), termination of photorefractoriness by alternate photoschedules has not been

evaluated. However, influence of long photoperiod in testicular activity during the preparatory and progressive phases has been assessed (Maitra,1987b). Information regarding photosensitivity/photostimulation or photorefractoriness is totally lacking in the tropical feral pigeons. However, the conjecture that the circannual variation in M secretion associated with the subtle variations in winter or summer solstice may be the modulating factor for testicular functions has been validated to a certain extent by the observations of testicular involution following pinealectomy or exogenous M administration accompanied by collateral alterations in the HHT and HHA axes (Patel et al.,1985; chapters I, III, V).Due to the lack of information regarding photoperiodic influences on reproductive functions in the feral pigeons, the current study has been undertaken to gauge the effect of long photoschedule on the activity of testes, adrenal and thyroid in both intact and PX birds in the early recrudescent phase. Since the testes enter into the progressive phase of activation in Jan, the birds have been exposed to the experimental photoschedule of LD 18:6 for 30 days, either in the month of Nov,or in the month of Dec.

Materials and Methods :

Procurement and maintenance of pigeons - as outlined in

chapter I.

Lighting and Lighting Schedules : The cages used for housing the experimental birds were illuminated using cool daylight flourescent tubes. The light intensity employed was of 2000 lux units which was measured with the help of lux-meter (Weston Electrical Instrument Corporation, NJ, USA). The cage temperature varied by only 1°C from that of the room temperature.

Experimental birds were exposed to two different photoschedules namely,

1. Normal light-dark (NLD or LD 12:12) -ie 12 hours of light followed by 12 hours of darkness.
2. Long photoperiod (LD 18:6) - ie 18 hours of light followed by 6 hours of darkness.

For LD 12:12 lights were switched on at 06.00 h and put off at 18.00 h whereas for LD 18:6 the lights were switched on at 06.00 h and put off at 24.00 h.

Experimental Set-ups :

Two similar sets of experiments were carried out just prior to the winter solstice ie during Nov.-Dec. and Dec.-Jan. For each of the experiments a total of 36 male pigeons were taken and divided into 6 groups and as per the set-up, the groups

were exposed to two different photoschedules for a period of 30 days. Two female pigeons were kept per group. As the experimental set-ups for both the study periods were similar, the common experimental set-ups are given below.

Group I (N 12:12) - This group of intact birds were exposed to NLD or LD 12:12 and served as intact controls.

Group II (N 18:6) -Intact birds were held under long photoschedule of LD 18:6 and served as experimental birds as well as controls against PX 18:6.

Group III (SPX 12:12) - Sham pinealectomised birds were held under NLD and served as sham control

Group IV (SPX 18:6) - Sham pinealectomized birds were held under the long photoschedule of LD 18:6 and served as sham control for PX 18:6.

Group V (PX 12:12) - These birds were pinealectomized and then exposed to NLD. This group served as experimental for N 12:12 as well as control against PX 18:6.

Group VI (PX 18:6) - Pinealectomised birds were held under the long photoperiod of LD 18:6 and served as experimental group.

As there was no significant difference between N 12:12 & SPX 12:12 and,N 18:6 & SPX

18:6, only the values of Group I and II are furnished herein.

Parameters and Methodology of evaluation : As outlined in chapter III.

Results :

Gravimetry : The testes of intact birds maintained under natural photoperiods had a low weight in the month of Dec., characteristic of the quiescent phase. However, in the month of Jan., the weight of testes tended to show an increase. In contrast, PX pigeons showed significantly decreased testes weights in both the months as compared to the intact birds. Though, the intact birds exposed to long photic schedules between Nov., and Dec., did not show any change in the testicular weight, the testes weight of birds exposed to long schedules between Dec., and Jan., showed significant increment. The long photoschedule had no effect on the weight of testes of PX pigeons in either of the two months.

Intact birds maintained under natural photoperiodism generally showed an increase in the weights of adrenal and thyroid from Dec., to Jan. This tendency of increase in the weight of adrenal and thyroid was further potentiated by exposure to long photoschedule. Similarly, PX as well as exposure of PX birds to long photoschedules also tended to

increase the weight of adrenal and thyroid. (Table 7.1; Fig 7A).

Histology :

Testis : There was progressive activation of the testis in intact birds between Dec.and Jan. Pinealectomy caused regressive changes in the testis of birds maintained either under natural photoperiod or experimental long schedules. The testis of intact birds of Dec.-Jan. group showed slightly enlarged tubules with early signs of activation as compared to the Nov.-Dec. group. Many of the tubules still contained degenerated germ cells though some of them were clear with spermatogonial activation. Interstitial cells still appeared regressed. The testis of PX birds in either month showed totally regressed tubules containing degenerating and vacuolated germ cells many of which depicted pyknotic nuclei. The basal layer of gonial cells was hypertrophied and the basement membrane was thick. Intersitial cells remained fibroblast like.

Intact birds exposed to LD 18:6 between Nov.and Dec.did not show any change in the testicular histology. However, the testis of birds exposed to LD 18:6 between Dec.-Jan. showed activated tubules many of which showed advanced stages of spermatogenesis. Elongated spermatids

Photic Schedule	Relative weight (mg/100g body wt.) Testes	Adrenal	Thyroid
N 12:12	27.37 ± 3.75	7.38 ± 0.74	5.06 ± 0.37
PX 12:12	20.45* ± 2.33	9.78* ± 1.02	7.55* ± 0.66
N 18:6	22.42* ± 3.61	7.96 ± 0.95	5.80* ± 0.72
PX 18:6	12.47* ± 1.01	9.48* ± 1.61	6.96* ± 0.65

Table 7.1a : Changes in relative weights of testes, adrenal and thyroid of intact and PX pigeons exposed to NLD or LD 18:6 in the months of Nov.-Dec. (* = Significant at $P < 0.05$; values are $\bar{x} \pm SD$)

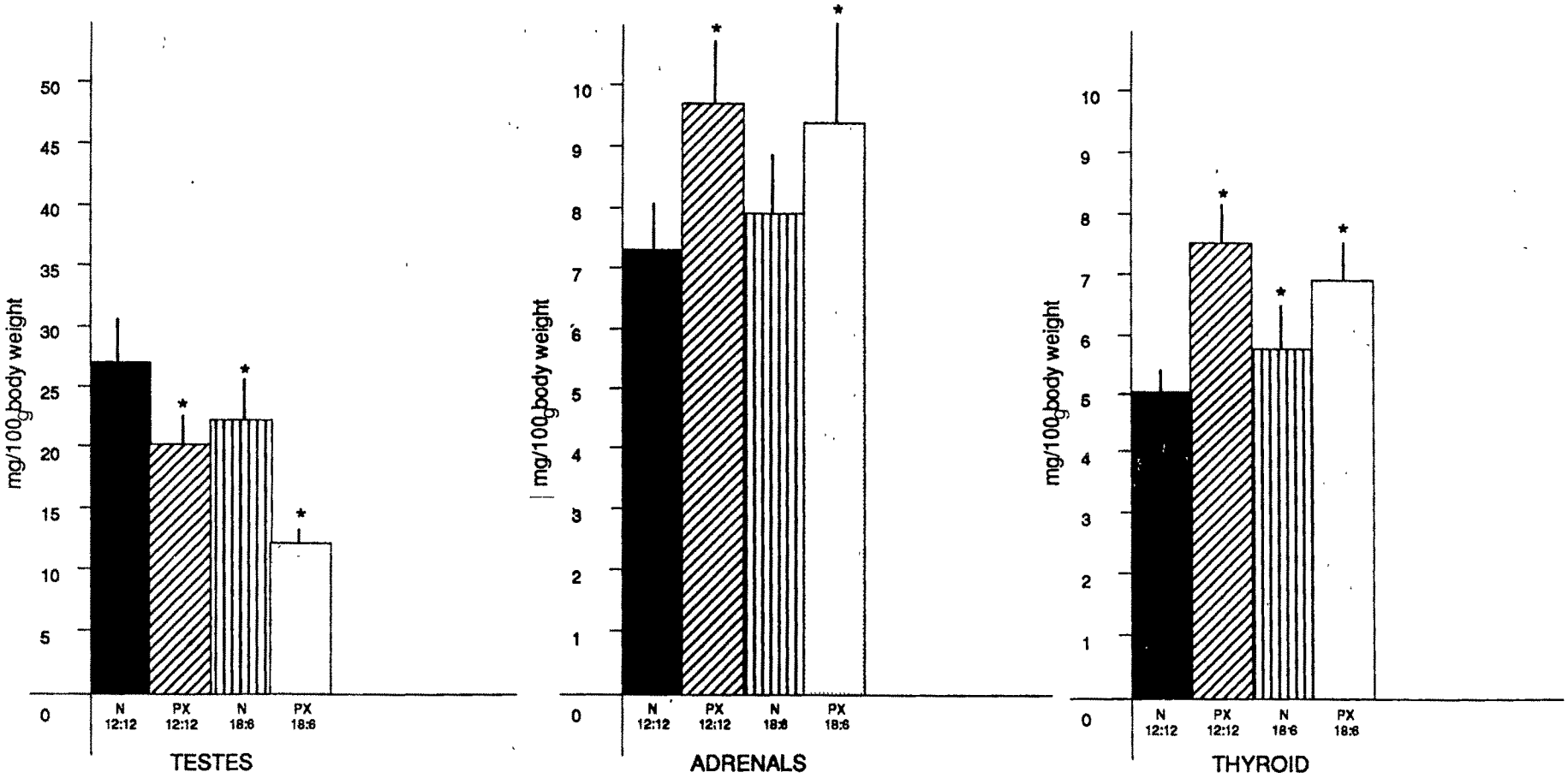


Fig. : 7 A : Changes in relative weight of testes, adrenals and thyroid of intact and PX pigeons exposed to NLD or LD 18:6 in the months of Nov. - Dec.
(* Significant at $P < 0.05$, values are $\bar{X} \pm$ SD).

Photic Schedule	Relative weight (mg/100g body wt.) Testes	Adrenal	Thyroid
N 12:12	40.56 ± 5.89	7.81 ± 1.40	5.09 ± 0.92
PX 12:12	21.07* ± 3.61	9.83* ± 1.45	6.72* ± 1.15
N 18:6	81.74* ± 9.97	8.66* ± 1.41	6.10* ± 0.97
PX 18:6	18.04 ± 2.89	9.40* ± 1.55	6.33* ± 1.04

Table 7.1b : Changes in relative weights of testes, adrenal and thyroid of intact and PX pigeons exposed to NLD or LD 18:6 in the months of Dec.-Jan. (* = Significant at $P < 0.05$; values are $\bar{X} \pm SD$)

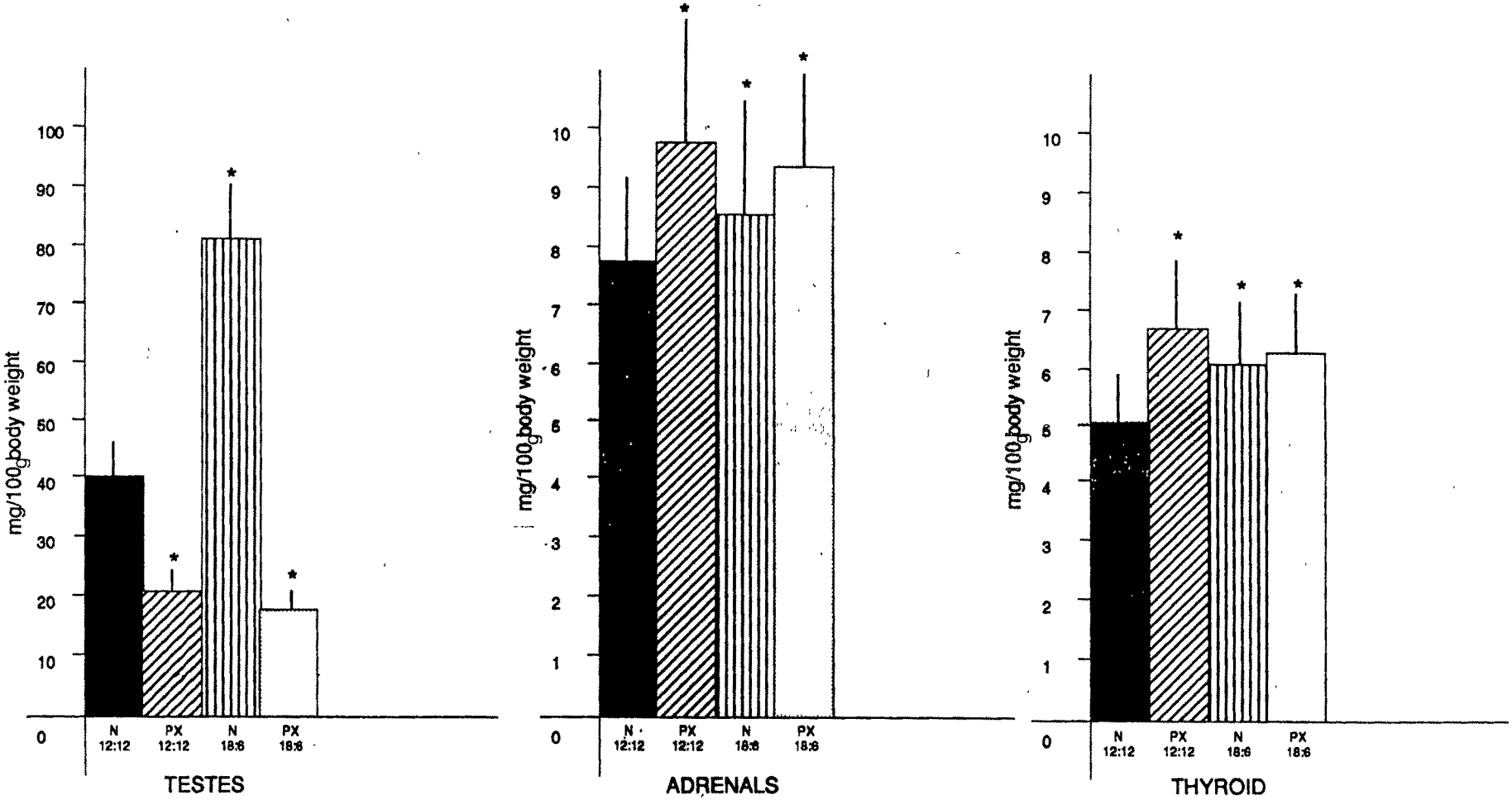


Fig. : 7 B : Changes in relative weight of testes, adrenals and thyroid of intact and PX pigeons exposed to NLD or LD 18:6 in the months of Dec. - Jan.
(* Significant at $P < 0.05$, values are $\bar{x} \pm SD$).

PLATE I

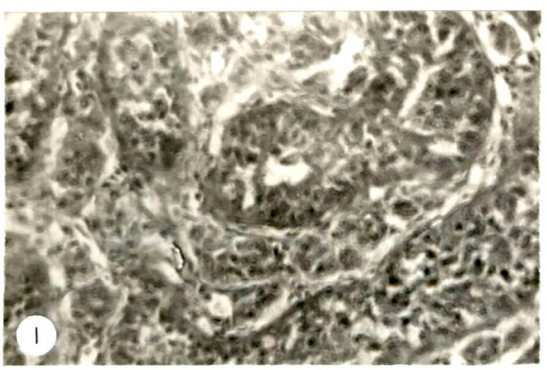
Figs 1-4 : Photomicrographs of testis of control and PX pigeons maintained under normal light and dark, just prior to the recrudescent phase (Dec-Jan).

Fig 1. : Tubules showing slight activation (400 X).

Fig 2. : Higher magnification of the same showing prominent interstitium (IC) (640 X).

Fig 3. : Tubules of PX pigeons with only degenerating cells (400 X).

Fig 4. : Higher magnification of the same (640 X). Note the sparse germ cells with pyknotic nuclei in the tubules and inactive fibroblast like inactive interstitium (IC).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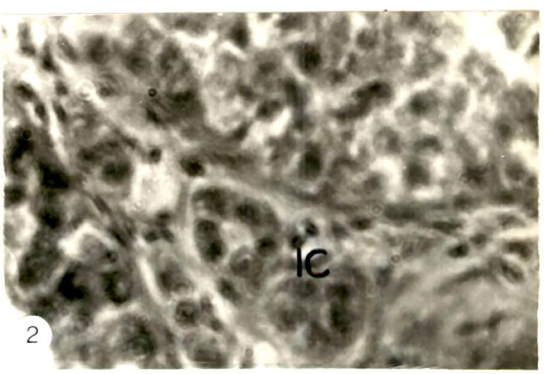
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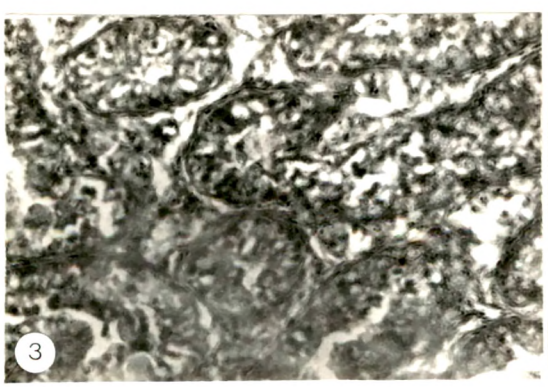
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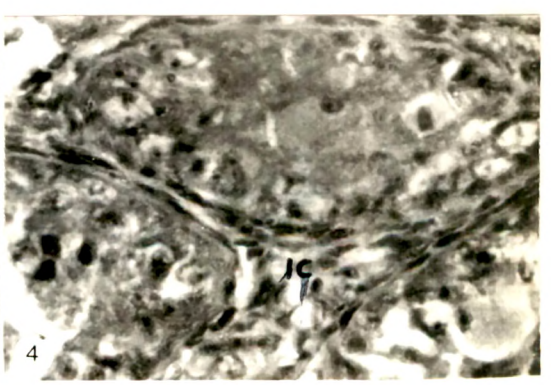
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PLATE II

Figs 5-10 : Photomicrographs of testis of control and PX pigeons exposed to LD 18:6 in the pre-recrudescent phase.

Fig 5. : Tubules of control bird showing commencement of spermatogenesis (400 X). Note the appearence of early meiotic cell types (arrow).

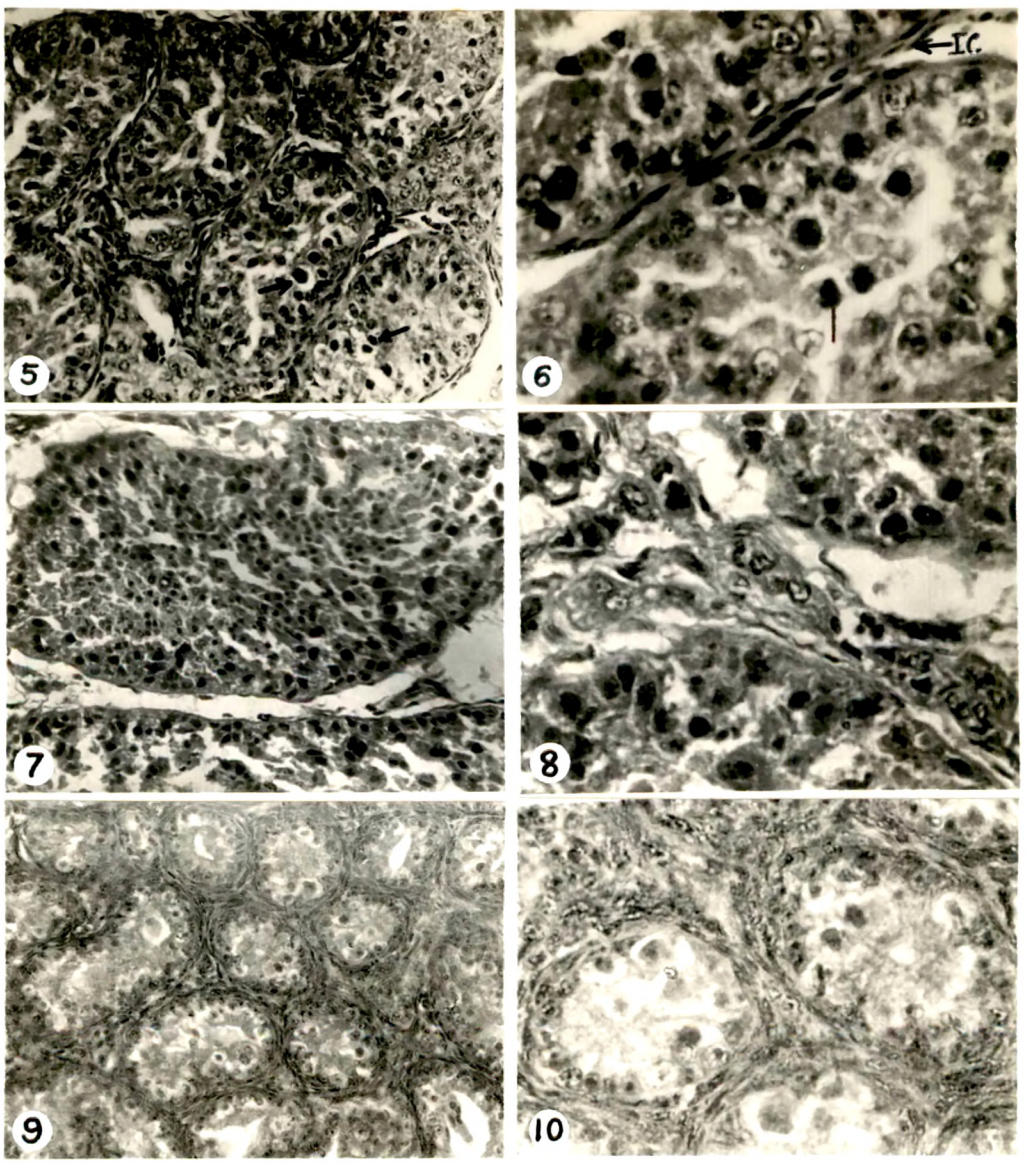
Fig 6. : Enlarged version of the same (640 X). Note the fibroblast like interstitial cells (IC).

Fig 7. : A single tubule showing various stages of spermatogenesis upto round spermatids (400 X).

Fig 8. : Higher magnification of the same showing prominent interstitial cells (640 X).

Fig 9. : Regressed inactive tubules of PX pigeon (400 X).

Fig 10. : Higher magnification of the same (640 X). Note the few basal spermatogonial cells and thickened basement membrane.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could be seen in some of the tubules. The interstitium showed varying degree of activation.

The testis of PX birds exposed to LD 18:6 either in the month of Nov.-Dec. or Dec.-Jan. remained inactive with shrunken tubules, thickened basement membrane and hypertrophied gonial cells. Most of the tubules showed degenerating germ cells while the interstitium remained highly involuted and regressed. (Plates I-II.)

Adrenal and Thyroid :

The thyroid of intact birds during Nov.-Dec. showed many follicles with depleted colloid content. The epithelial cell height was moderately high. The adrenal at the same time showed regressed cortical tissue. During Dec.-Jan. the thyroid had a mixed population of follicles with or without colloid. The follicular epithelium was low cuboidal. The adrenal showed more active cortical cords. The thyroid and adrenal of PX birds in the both periods showed a similar condition of thyroid follicles depleted of their colloid content and regressed adrenocortical tissue. Intact birds exposed to LD 18:6 in Nov.-Dec. did not show much changes while the Dec.-Jan. group of birds showed significant changes in adrenal and thyroid. Thyroid was marked by colloid filled follicles lined by low cuboidal epithelium while the adrenal

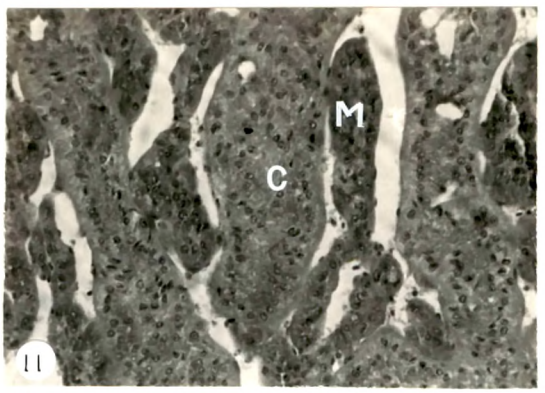
PLATE III

Figs 11-13: Photomicrographs of adrenal of intact and PX pigeons exposed to NLD and LD 18:6 in the recrudescent phase. (200 X).

Fig 11. : Adrenal of intact bird exposed to NLD showing cortical and medullary cords.

Fig 12. : Adrenal of PX bird exposed to NLD showing involuted cortex.

Fig 13. : Adrenal of intact bird exposed to LD 18:6 showing increased cortico-medullary ratio. Note the active state of the cortical cells.



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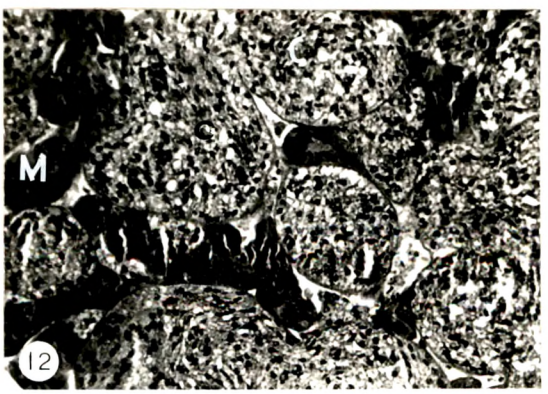

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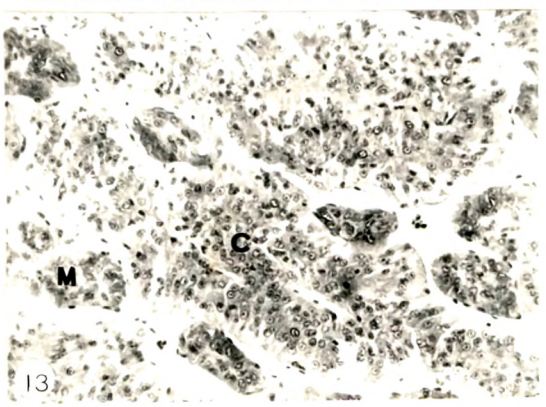

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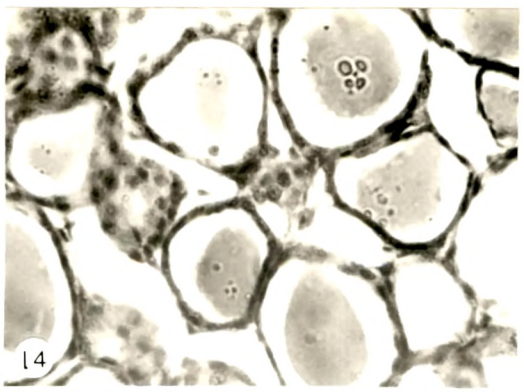
PLATE IV

Figs 14-16: Photomicrographs of thyroid of intact and PX pigeons exposed to NLD and LD 18:6 in the recrudescent phase. (400 X)

Fig 14. : Thyroid of intact bird exposed to NLD showing lesser colloid content.

Fig 15. : Thyroid of PX bird exposed to NLD showing loss of colloid from the follicles.

Fig 16. : Thyroid of intact bird exposed to LD 18:6. Note the increased colloid content of the follicles.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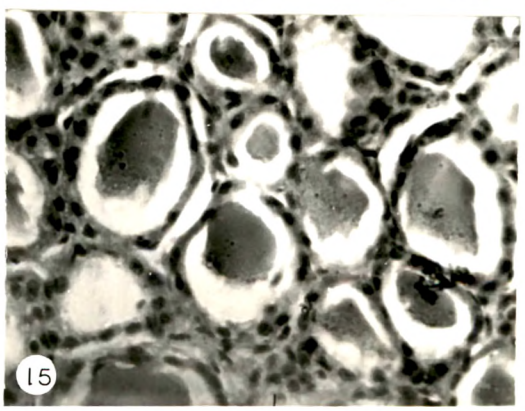
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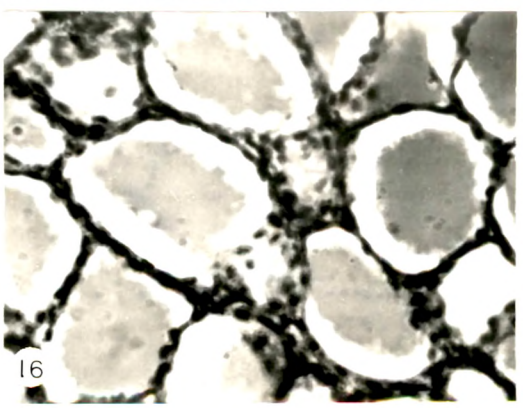
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	SERUM HORMONES		
	T4 (ng/ml)	T3 (ng/ml)	B (ug/dl)
N 12:12	17.33 ± 1.75	3.98 ± 0.71	7.83 ± 0.86
PX 12:12	21.66 ± 2.07	3.74 ± 0.91	6.12 ± 0.81
N 18:6	15.32* ± 1.89	2.04* ± 0.59	8.04* ± 0.76
PX 18:6	20.81 ± 2.11	3.43 ± 0.64	6.31 ± 0.61

Table 7.2a : Changes in serum levels of T4, T3 and corticosterone(B) in intact and pinealectomised pigeons exposed to NLD or LD 18:6 in the months of Nov.-Dec.
(* = Significant at $P < 0.05$; values are $\bar{x} \pm SD$)

	SERUM HORMONES		
	T4 (ng/ml)	T3 (ng/ml)	B (ug/dl)
N 12:12	13.08 ± 1.12	2.11 ± 0.41	8.26 ± 0.91
PX 12:12	20.87 ± 1.82	3.58 ± 0.74	6.34 ± 0.55
N 18:6	12.11* ± 1.00	1.38* ± 0.06	8.99* ± 0.76
PX 18:6	19.46 ± 1.01	3.10 ± 0.52	6.41 ± 0.71

Table 7.2b :Changes in serum levels of T4, T3 and corticosterone (B) in intact and pinealectomised pigeons exposed to NLD or LD 18:6 in the months of Dec.-Jan.
(* = Significant at $P < 0.05$; values are $\bar{x} \pm SD$)

showed active hypertrophied cortical tissue. Medullary tissue on the other hand was regressed to a noticeable extent. Pinealectomised pigeons exposed to long photoschedules, either in Nov.-Dec. or Dec.-Jan. did not show any change in their thyroids or adrenals. The thyroid had mostly empty follicles devoid of colloid while the adrenal showed regressed cortical tissue. (Plates III, IV).

Serum T4, T3 and B levels : Serum T4 and T3 of Dec.-Jan. group of intact birds were decreased as compared to the Nov.-Dec. group of birds while reverse was the case for serum B levels. Pinealectomised birds in both periods showed relatively higher T4 & T3 levels and decreased B level compared to the corresponding intact controls. Whereas PX birds exposed to long photic schedule did not show any change in the serum levels of these hormones in either period, intact birds exposed to long photic schedules registered an increase in serum B level and a decrease in serum T4 and T3 level,more pronounced in the Dec.-Jan. group of birds (Table 7.2a,b).

DISCUSSION :

Seasonal reproductive activities in most of the temperate species of birds are known to be driven by photoperiodic variations. In these birds gonadal activity is induced by

increasing daylength leading to full gonadal functions in the period of long days, followed by sudden collapse of gonads, inspite of the day lengths being longer in many cases and finally reach a quiescent phase during which the birds enter into a photo-insensitive phase referred to as photo refractoriness (Kumar and Kumar, 1992). Such cycles of photo-inducibility and photorefractoriness are now known to occur even in many tropical and sub-tropical species of birds(Singh and Chandola,1982; Maitra,1987; Kumar and Kumar, 1991;1993). Though the occurrence of photorefractoriness in tropical feral pigeons is not known,the present study was principally designed to see whether these birds exhibit any stimulatory influence vis-a-vis gonadal recrudescence when exposed to long photic schedules during the end of their quiescent phase and also to test the effect of pinealectomy on the same.The end period of quiescent phase was specifically chosen as, the post-regressive phase and the mid-quiescent phase are known to be photo-insensitive in most of the birds. The results obtained suggest that this species is sensitive to light as the birds maintained in a long photoperiod depicted testicular enlargement and spermatogenesis as compared to birds maintained under the normal photoschedule. Moreover, the stimulatory changes were more marked in the Dec.-Jan. group of birds with none in the Nov.-Dec. group of birds. This suggests that photo-sensitivity /photoinducibility occurs only towards the

end of a quiescent phase and prior to that the birds are probably in a state akin to that of photorefractoriness. The hastening of testicular recrudescence under the long photoperiod also agrees well with the reproductive activation of these birds following the winter solstice and the peak of the reproductive activities coinciding with the long summer days (March - May). Another significant revelation is the inability of PX birds to show normal gonadal recrudescence either under normal photo-periodic conditions or long photic-schedule. Apparently, PX can inhibit the activation of the HHG axis atleast in the immediately ensuing breeding phase. Literature on the involvement of the pineal in the photoperiodic control of reproductive activities in birds is not very enviable, as results obtained in pinealectomy experiments are highly varied. In this respect, while Balasubramanian and Saxena (1973) reported precocious recrudescence after PX in the Indian weaver birds, Haldar and Ghosh (1990) showed inhibition of testicular recrudescence in the Indian jungle bush quail and Wilson (1991) demonstrated uninhibited gonadal recrudescence after PX in the American tree sparrows. The present observation is more in agreement with that of Haldar and Ghosh. By an elegant series of long duration experiments, Wilson (1991) demonstrated extaocular, extrapineal encephalic photoreception-mediated gonadal functions in the American tree sparrows. The present experiments though show an inhibitory influence of PX in the

immediately ensuing gonadal cyclicity, do not however, discount the possibility of alternative mechanisms of photoreception and resultant activation of gonadal cyclicity occurring later.

Other observations pertain to HHA and HHT axes and in general, PX pigeons depicted hyperactive HHT axis and suppressed HHA axis. In contrast, photo-stimulated birds show suppressed HHT axis and activated HHA axis. Obviously,long photoperiod-induced testicular activation is accompanied by concurrent activation of the adrenocortical functions and suppressed colloidal release from the thyroid. The changes in the serum levels of T4, T3 on one hand and of B on the other hand are fully supportive of this conjecture. The present observations are in total conformity with the earlier findings of PX or M-induced alterations in HHT or HHA axis (chapters I, III and V). Based on the findings in the above studies, it was inferred that while PX-induced testicular regression is due to the activation of HHT axis leading to increased T4 secretion, the exogenous M-induced testicular regression is due to a direct inhibition of HHG axis inspite of a favourable suppression of HHT axis.

It can be concluded from the present observations that a high effective threshold level of M attained closer to the period of winter solstice suppresses the HHT axis thereby providing

a favourable situation for testicular recrudescence. The subsequent testicular recrudescence occurs due to the stimulation of HHG and HHA axis due to the increasing day-lengths subsequent to winter solstice. The differential changes affecting the HHT axis on one hand and HHG and HHA axes on the other can apparently be hastened by exposure of the birds to long photoperiod towards the end of the quiescent phase.