CHAPTER IX

Photoperiod-Adrenal interactions on testicular recrudescence in the feral pigeon, Columba livia.

Seasonal changes in the adrenocortical activity in relation to gonadal cyclicity have been studied in some wild species of birds (Assenmacher,1973; Holmes and Phillips, 1976; Silverin, 1979;Thapliyal,1981; Peczely,1985). The adrenal-gonad relationship has been reported to be either parallel (Fromme-Bouman,1962; Bhattacharya and Ghosh,1965; Moens and Coessens,1970; Smith and Brereton,1976; Thapliyal,1981), or inverse (Hartman,1946; Lorenzen and Farner,1964; Dusseau and Meier,1971; Silverin,1979) or out of phase (Chaturvedi and Thapliyal,1979). There are also some reports suggesting involvement of adrenocortical activity in the mediation of photoperiodic response of gonads (Meier et al.,1971, Meier and Dusseau,1973; Wilson and Follet,1975; Johnson and vanTienhoven,1981). Previous studies from this laboratory have demonstrated parallel adrenal-testes as well as pineal-adrenal-testes relationships in feral pigeons (Patel et al.,1985, 1987; Ayyar et al.,1992). Further, it was also shown that increased photoperiod in the pre-recrudescent phase can stimulate the activity of both testes and adrenal (Chapter VII). In this context, it becomes pertinent to evaluate photoperiod-adrenal interactions in terms of gonadal

cyclicity. However, no investigations of this nature have been carried out in birds, though there are a few reports involving photoperiod-thyroid interactions.

The present study has been designed to this end and has tried to evaluate the response of testes and thyroid in pigeons subjected to functional manipulation of adreno-cortical activity and exposed to either normal light/dark cycle (NLD, LD 12:12) or a long photoperiod (LD 18:6) in the pre-recrudescent phase.

Materials and Methods :

Procurement and maintenance of pigeons - as outlined in chapter I. Lighting and lighting schedules - as outlined in chapter VII.
Parameters and methodology of evaluation - as in chapter I.

Preparation of Solutions :

Corticosterone (CORT) :

Corticosterone procured from Sigma Chemicals, St. Louis, U.S.A. was used for the experiment. The required quantity of the same was first dissolved in 0.5 ml propylene glycol and then made upto required concentration with 0.9% sterile

saline. For the experiment,dosage of 2 ug/0.5 ml saline was used. The solution was stored in the refrigerator for daily use.

Dexamethasone (DXM) :

DEXONA (Dexamethasone sodium phosphate) Cadila Laboratories, Ahmedabad, India was used. The required concentration was acquired by diluting it with 0.9% sterile saline and stored in the refrigerator for daily use.

Experimental Set-ups :

In the month of Jan. a total of 36 male pigeons having uniform gonadal condition were divided into 6 groups. Two female pigeons were added to each group.

Group I (N 12:12) :Intact pigeons were exposed to LD 12:12 and served as control for CORT 12:12 and DXM 12:12.

Group II (CORT 12:12) :These birds were given daily injections of corticosterone at 09.00 h and exposed to LD 12:12.

Group III (DXM 12:12):These birds were given daily injection

of DXM at 17.00 h and exposed to LD 12: 12.

Group IV (N 18:6) :Intact birds were exposed to LD 18:6 and served as control for CORT18:6 and DXM 18:6

Group V (CORT 18:6) :These birds were given daily injection of corticosterone at 09.00 h and exposed to long photo-period of LD 18:6

Group VI (DXM 18:6) :These birds were given daily injections of DXM at 17.00 h and exposed to LD 18:6

Results :-

Gravimetric changes : (Table 9.1; Fig 9A)

The weight of testes in birds maintained under NLD was low.Treatment with dexamethasone (DXM) or corticosterone (CORT) further decreased the weight. Birds maintained at LD 18:6 showed increased testes weight but simultaneous treatment with either DXM or CORT tended to attenuate the weight increase. Both the thyroid and adrenals showed

Treatments	Relative weight (mg/100g body weight) Testes	Adrenal	Thyroid
N 12:12	34.69 ± 7.81	7.58 ± 0.93	5.18 ± 0.25
N 18:6	70.58 ± 9.56	8.32 ± 0.50	6.29 ± 0.80
CORT 12:12	21.07* ± 2.70	8.76* ± 0.61	8.14* ± 0.92
CORT 18:6	28.97* ± 2.57	7.65* ± 0.66	6.14 ± 0.73
DXM 12:12	18.44* ± 1.72	9.40* ± 1.07	6.00* ± 0.41
DXM 18:6	47.05* ± 2.89	7.24 ± 0.81	9.83* ± 1.05

Table 9.1 :Changes in relative weight of testes,adrenal and thyroid of pigeons treated with CORT or DXM and exposed to NLD or LD 18:6 in the pre-recrudescent phase .
(* = Significant at $P < 0.05$; values are $\bar{x} \pm SD$)

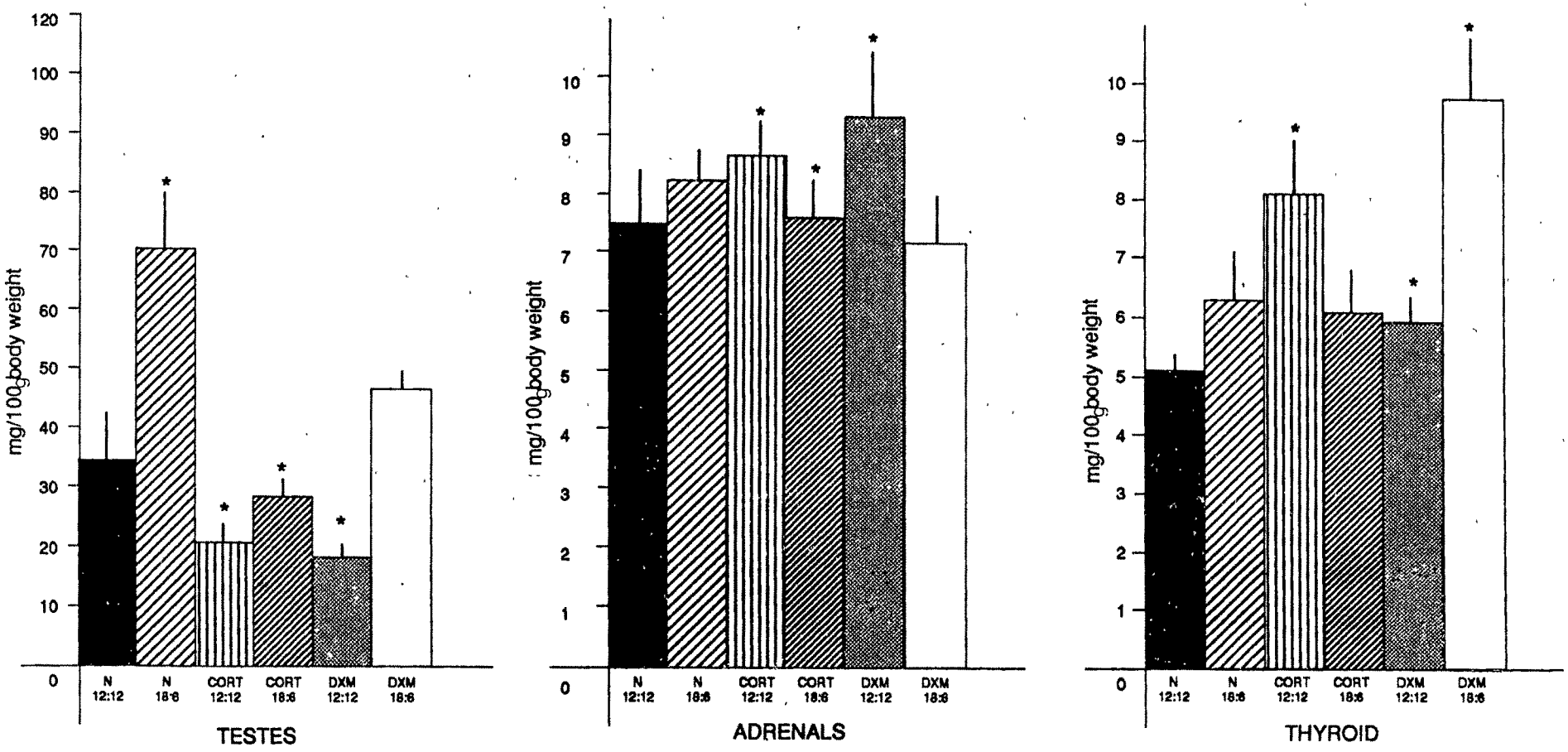


Fig. : 9A Changes in relative weight of testes, adrenals and thyroid of pigeons treated with CORT or DXM and exposed to NLD or LD 18:6 in the pre-recrudescent phase.
(* = Significant at $p < .05$; values are $\bar{x} \pm SD$).

increased weight in birds treated with either DXM or CORT and maintained under NLD. Exposure to LD 18:6 also increased the weight of both the glands as compared to NLD. However, the weight of the adrenals did not show any change when treated with either DXM or CORT. In contrast, under LD 18:6, DXM treatment increased thyroid weight maximally while CORT treatment did not show much change.

Histological Changes :

Testes : The testes of pigeons maintained under NLD showed early signs of activation. Tubules were slightly enlarged and the lumen of many tubules contained degenerating germ cells. Some of the tubules were clear of degenerated germ cells and showed signs of initiation of spermatogenesis. Interstitial cells were generally regressed though at some places they showed signs of activation. The testis of DXM treated birds remained inactive with many of the tubules showing single layered hypertrophied gonial cells. Interstitium was inactive and fibroblast like. The tubules of CORT treated birds were also like those of control birds. Signs of germcell recovery was noticeable in some tubules and were relatively better organised. Interstitial cells depicted hypertrophy and hyperplasia. Testes of birds exposed to LD 18:6 showed active seminiferous tubules with many of them depicting advanced stages of spermatogenesis marked by the presence of elongated

PLATE I

Figs 1-6 : Low magnification photomicrographs of testis of control and DXM and corticosterone treated pigeons under NLD and LD 18:6 photoperiodic schedules in the pre-recrudescent period. (200 X).

Fig 1. : Testis section of control bird exposed to NLD. Note the small tubules with large interstitial spaces.

Fig 2. : Testis section of DXM treated bird exposed to NLD. Note the highly involuted tubules.

Fig 3. : Testis section of corticosterone treated bird exposed to NLD. Tubules though small show signs of activation.

Fig 4. : Tubules of control birds exposed to LD 18:6. Note the enlarged tbules with many spermatogenic stages.

Fig 5. : Tubules of DXM treated birds exposed to LD 18:6. Note the relatively smaller tubules and fewer spermatogenic stages.

Fig 6. : Tubules of corticosterone treated bird exposed to LD 18:6. Note the relatively smaller tubules but with spermatogenic stages.

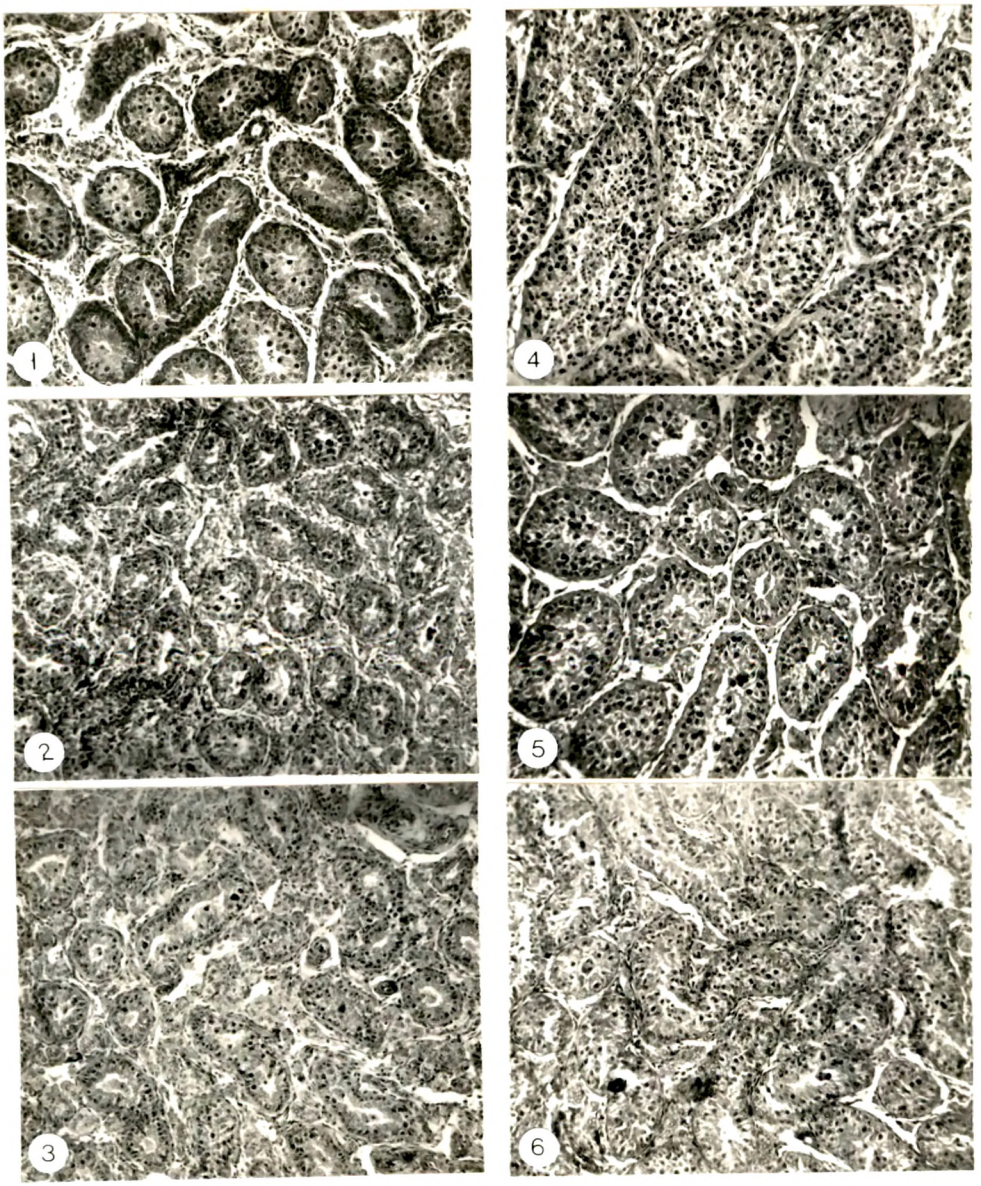


PLATE II

Figs 7-12 : Higher magnification photomicrographs of testis of control and DXM and corticsterone treated pigeons under NLD and LD 18:6 photoperiodic schedules in the pre-recrudescent period (400 X)

Fig 7. : Tubules of control birds exposed to NLD showing initiation of spermatogenesis. Note the few early meiotic cell types and mixed population of inactive (&) and active (*) interstitial cells.

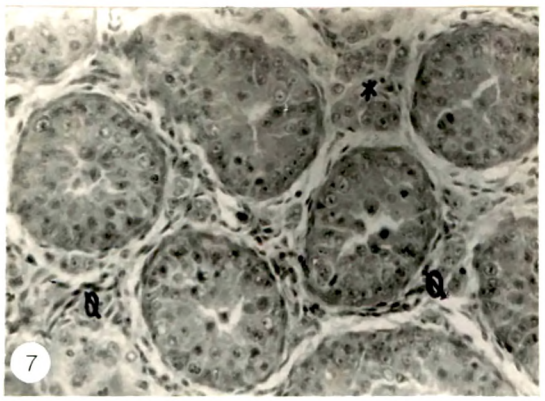
Fig 8. : Tubules of DXM treated bird exposed to NLD. Note the regressed inactive tubules with degenerating germ cells.

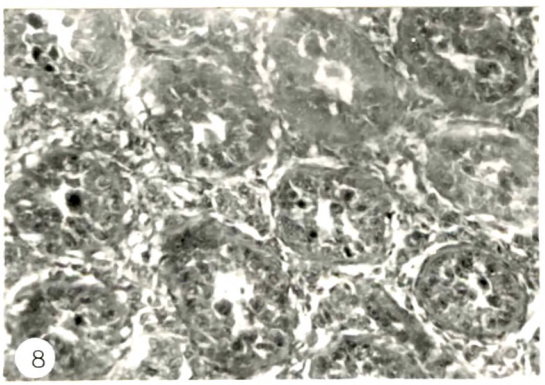
Fig 9. : Tubules of corticosterone treated bird exposed to NLD.

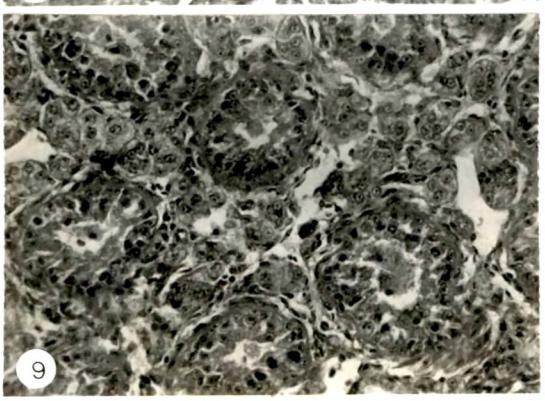
Fig 10. : Enlarged tubules of bird exposed to LD 18:6 showing all stages spermatogenesis upto spermatids.

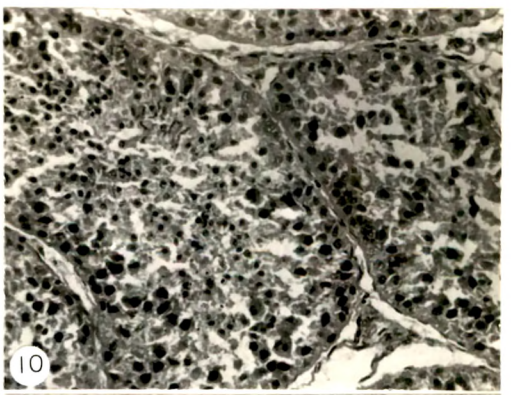
Fig 11. : Tubules of DXM treated bird exposed to LD 18:6 showing only sparse early meiotic stages.

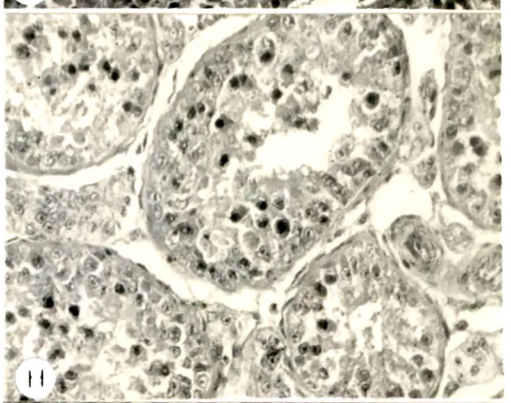
Fig 12. : Tubules of cortiocosterone treated bird exposed to LD 18:6 showing more members of meiotic cell types.


7


8


9


10


11

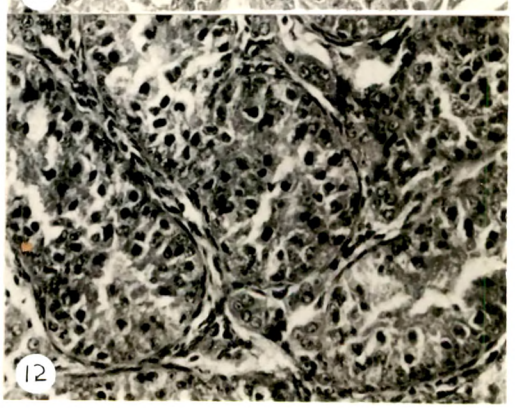
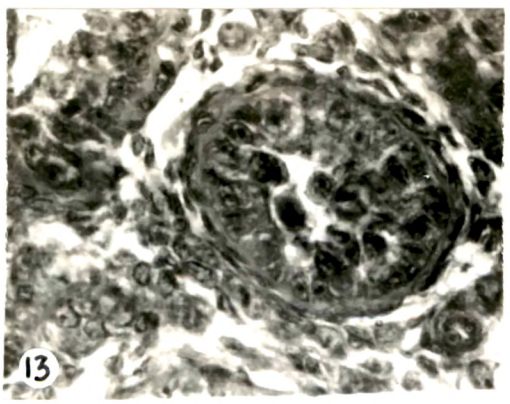

12

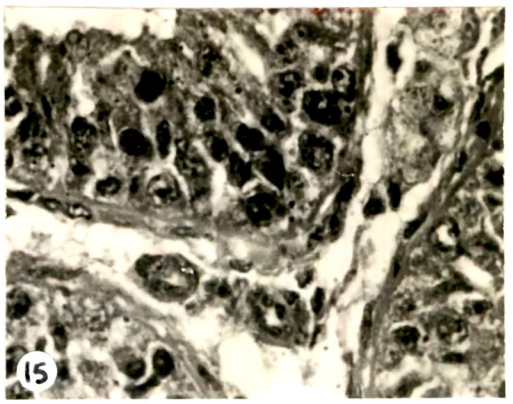
PLATE III

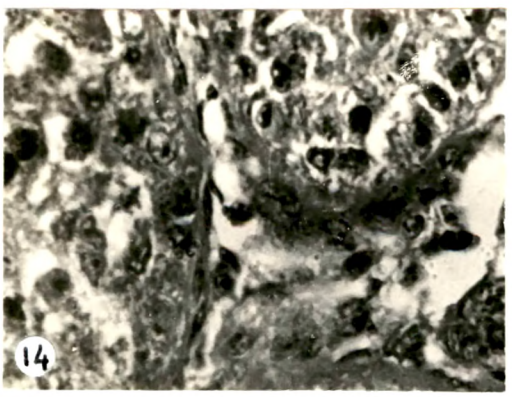
Figs 13-16: Photomicrographs of testis of DXM treated pigeons exposed to NLD and LD 18:6 in the pre-recrudescent phase (640 X).

Fig 13. : Testis section of bird exposed to NLD showing regressed tubules with hypertrophied germ cells and pyknotic nuclei.

Figs 14-16: Testis section of bird exposed to LD 18:6. showing tubules with few early meiotic stages. Note the mixed population of interstitial cells, small to hypertrophied.


13


15


14

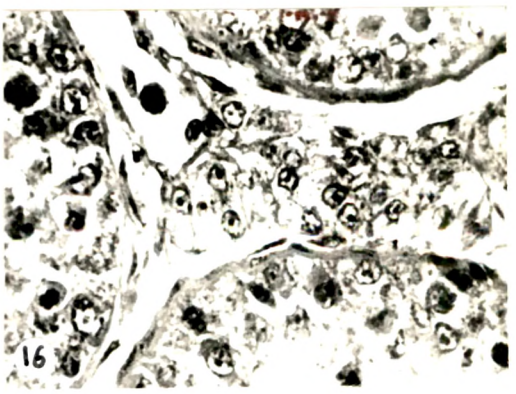
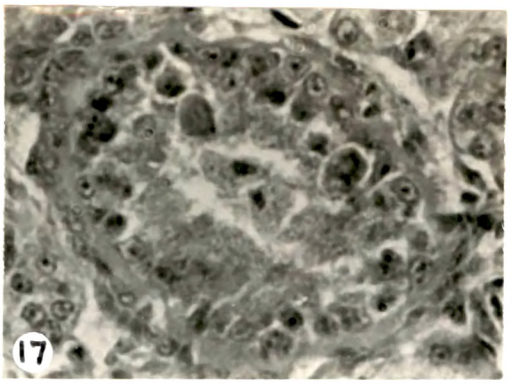

16

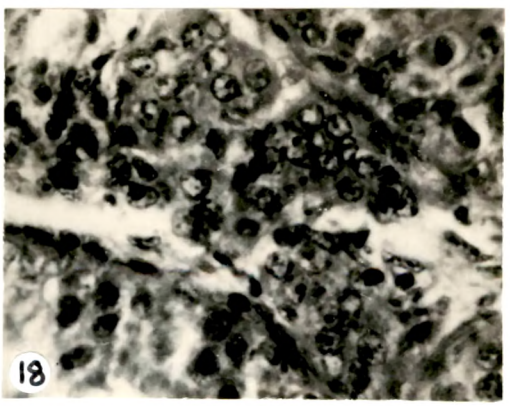
PLATE IV

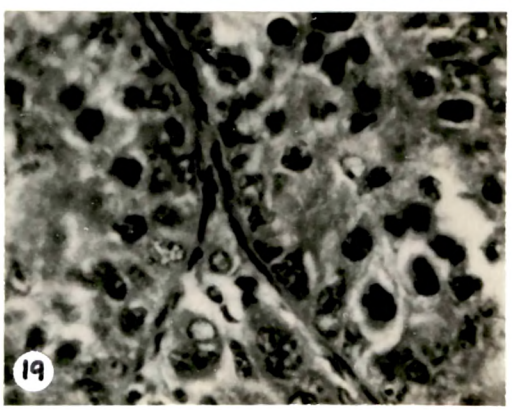
Figs 17-19: Photomicrographs of testis of corticosterone treated pigeons exposed to NLD and LD 18:6 in the pre-recrudescent phase. (640 X).

Fig 17. : A single tubule of bird exposed to NLD showing only spermatogonial cells. Note the well formed interstitial cells.

Figs 18-19: Testis sections of bird exposed to LD 18:6 showing hyperplastic interstitium (18) and tubules with many meiotic germ cells and hypertrophied interstial cells (19).


17


18


19

spermatids. Interstitium showed various degrees of activation ranging from fibroblast like to well formed cells. The testes of DXM treated birds also showed initiation of spermatogenesis but the tubules showed only fewer meiotic stages. Post-meiotic stages were not seen. Overall, the tubules showed various grades of activation. Interstitial cells constituted mixed population of well formed to fibroblast like cells. The tubules of CORT treated birds were also active and they were also devoid of spermatids. Many meiotic cells were clearly visible. The interstitium showed marked hypertrophy and hyperplasia. (Plates I - IV)

Adrenal : The adrenal of NLD birds showed greater cortico-medullary ratio and the cortical cords appeared active. Dexamethasone treatment was marked by cortical regression with reduced cortico-medullary ratio. Corticosterone (CORT) treatment induced cortical hypertrophy, and the medulla appeared regressed. The overall cortico-medullary ratio was high. Exposure to LD 18:6 also caused medullary regression while the cortex appeared prominent and hypertrophied. Treatment with DXM induced marked cortical regression as in the case of NLD. Treatment with CORT amplified the LD 18:6 changes with prominent cortical hypertrophy and medullary regression. (Plate V)

Thyroid : Thyroid of birds maintained under NLD showed a

PLATE V

Figs 20-25: Photomicrographs of adrenal of control and DXM and corticosterone treated pigeons exposed to NLD and LD 18:6 in the pre-recrudescent phase (200 X).

Fig 20. : Adrenal of control bird exposed to NLD showing cortical and medullary cords.

Fig 21. : Adrenal of DXM bird exposed to NLD. Note the regressed cortical cords.

Fig 22. : Adrenal of corticosterone treated bird exposed to NLD. Note the enlarged and active state of the cortex.

Fig 23. : Adrenal of control bird exposed to LD 18:6. Note the greater cortical area as compared to NLD (fig 20).

Fig 24. : Adrenal of DXM treated bird exposed to LD 18:6. Note the regressive changes in the cortical tissue.

Fig 25. : Adrenal of corticosterone treated bird exposed to LD 18:6. Note the highly enlarged state of cortex.

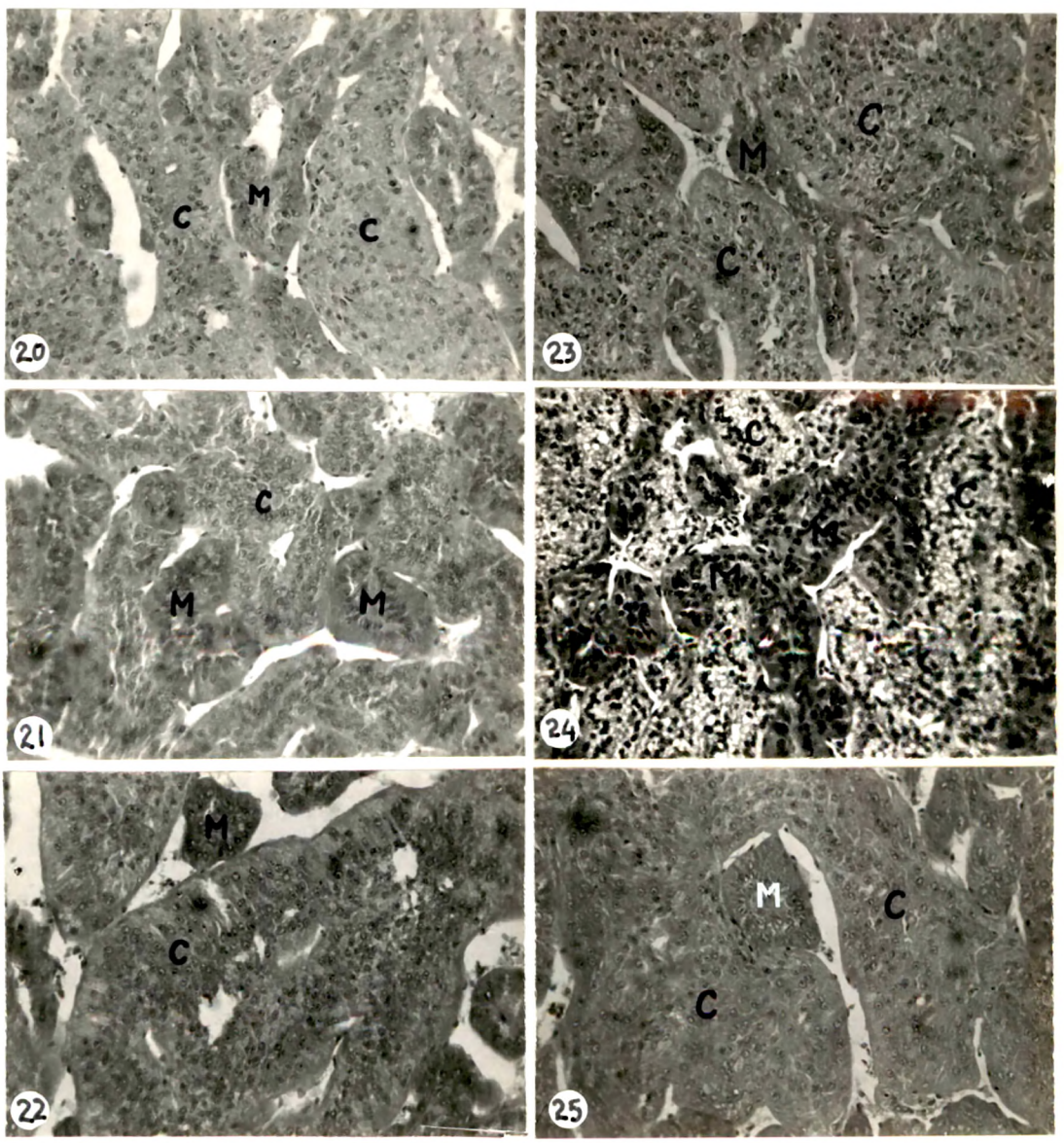

C
M
C
20
M
C
C
23
C
M
M
21
C
M
C
M
24
M
C
C
22
M
C
25

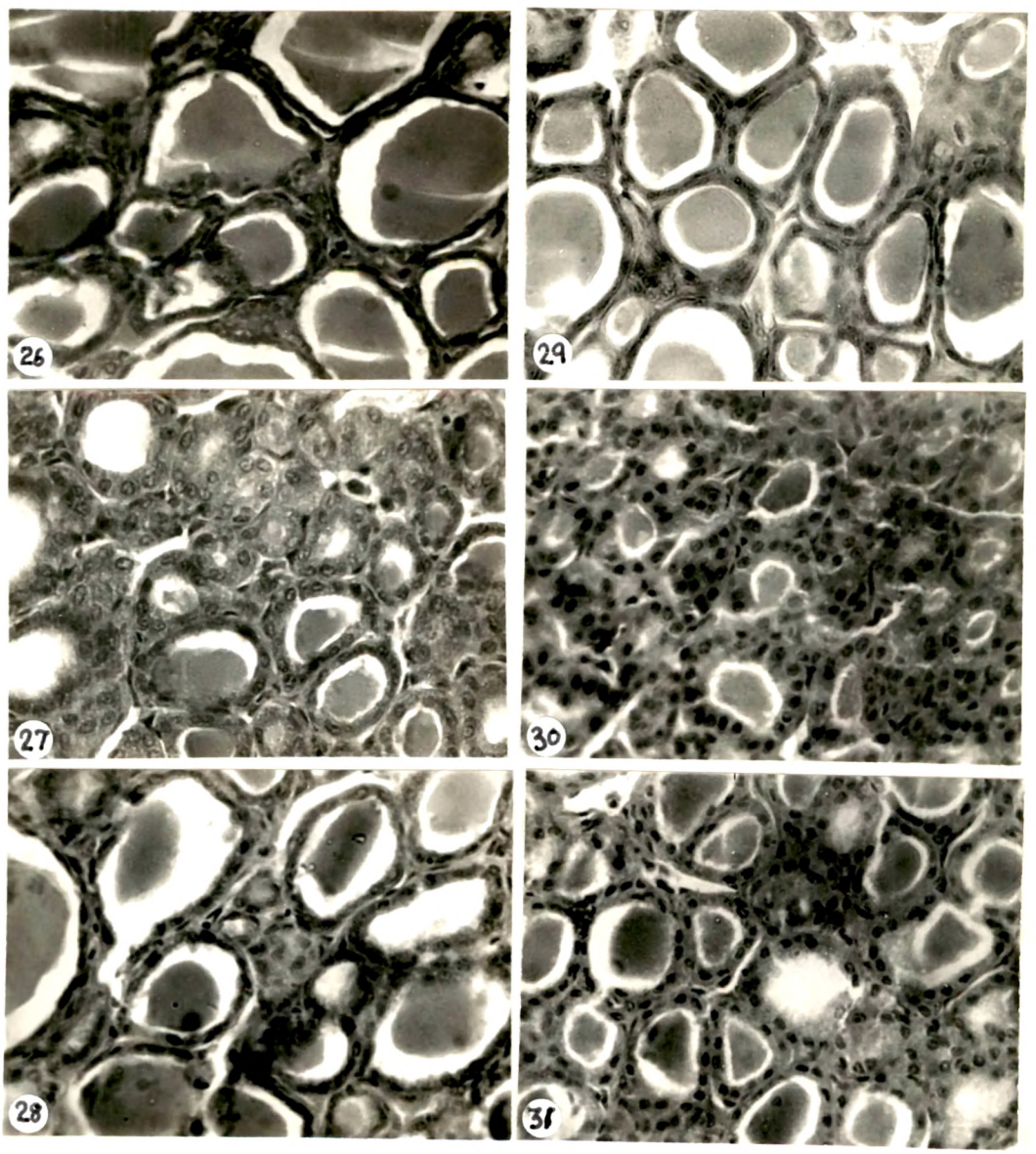
PLATE VI

Figs 26-31: Photomicrographs of thyroid of control and DXM and corticosterone treated pigeons exposed to NLD and LD 18:6 in the pre-recrudescent phase (400 X).

Fig 26,29 : Thyroid of control bird exposed to NLD and LD 18:6 respectively showing follicles with near full colloid.

Fig 27,30 : Thyroid of DXM treated birds exposed to NLD and LD 18:6 respectively showing follicles with depleted colloid content and hypertrophied epithelium. Note the potentiating effect of LD 18:6.

Fig 28,31 : Thyroid of corticosterone treated birds exposed to NLD and LD 18:6 respectively showing follicles with depleted colloid content and hypertrophied epithelium. Note the potentiating effect of LD 18:6.


26
29
27
30
28
31

mixed population of colloid filled and empty follicles. The follicular epithelium was cuboidal to flat. The follides of DXM treated birds appeared hyperactive. The epithelium was highly hypertrophied with the result the lumen of many follicles were obliterated. In contrast, the follicles of CORT treated birds appeared shrunken. Many of them showed depleted colloid content and the epithelium remained cuboidal to flat. Thyroid of birds exposed to LD 18:6 showed normal looking follicles. Many of the follicles were full of colloid and the epithelium was cuboidal to low cuboidal. Treatment with DXM showed a similar hyperactive state as in NLD. Epithelium was hypertrophied and the lumen of many follicles was obliterated. Even CORT treated birds depicted hypertrophy and hyperplasia of the epithelial cells; many of the follicles had narrow lumen and low colloid content.(Plate VI)

DISCUSSION :

Increasing day length is known to activate the gonads of many seasonally breeding temperate species of birds (see Kumar & Kumar,1991; Thapliyal,1993). Even some of the tropical and subtropical species of birds are also reported to synchronise their reproductive activities with the annual variations in photoperiodism (Singh and Chandola,1982; Maitra,1987; Kumar and Kumar,1991, 1993). Previously, it was shown that in the subtropical feral pigeons, testicular

activation can be hastened under a long photoschedule in the immediate pre-recrudescent phase (Chapter VII). An earlier study from this laboratory had shown testicular involution in response to adrenocortical suppression thereby suggesting a parallel adrenal-gonad axis (Ayyar et al.,1992). The present observations on increased testicular weight and functional activation in LD 18:6 exposed birds confirms the earlier observations (Chapter VII). Though it was previously shown that induced hypocorticalism brings about testicular involution in the breeding season, and hypercorticalism in the regression phase partly activates the regressed testes (Ayyar,1987; Ayyar et al.,1992), the present investigations involving functional manipulation of adrenocortical activity in relation to two different photoschedules (NLD & LD 18:6) on testicular recrudescence have provided evidences for intricate inter-relationships.

The present observations indicate adreno-cortical activation under the long photoperiod coinciding with hastened testicular recrudescence. Obviously, long photoperiod exerts a stimulatory action on the HHG axis as well as the HHA axis. The only study addressed to understand the inter-relationship between adreno-cortical activity and photoperiodic and testicular cycles in the parakeet has also shown a positive relationship between photoperiod, adreno-cortical activity and testicular functions (Maitra,1987). Despite the fact that

there is a parallel relationship between adrenal and testes in the feral pigeons, both induced adrenocortical insufficiency by DXM as well as adrenocortical excess by CORT retarded testicular recrudescence under NLD. Similar retardatory influence, of both adrenocortical insufficiency as well as excess on the hastened testicular recrudescence occurring under the long photoperiod, was also evident.

Earlier studies have clearly established the suppressive effect of T4 on testicular functions in the pigeons (Chapter I, III). Interestingly, in the present investigations, both DXM and CORT treatments caused hyperactivity of the thyroid. Obviously, the suppressive effects of both DXM and CORT on testicular activation is mediated through the increased thyroid hormone output. In this respect the inhibitory effect of thyroid hormones on gonadal functions is recognised in many birds (Peczely,1985; Lea et al.,1986; Pathak and Chandola,1982). In the light of the known inverse functional relationship between adrenal and thyroid in the pigeon (Patel et al.,1985; Ayyar et al.,1992), though the DXM -induced thyroid activation is understandable, the concurrent CORT-induced activation is a bit perplexing. The possibility of both DXM and CORT stimulating the HHT axis by their action at a common site is the only logical explanation. It is worth recalling that specific temporal phase relationship between CORT and prolactin (PRL) or even between brain serotonergic

and dopaminergic activities have been purported to play an important role in the timing of seasonal reproductive activities and lipid metabolism in birds and mammals (see Meier and Cincotta,1993). A distinct possibility in this context is that the administration of CORT in the present experimental set-up may have struck an unfavourable phase relationship leading to HHT axis activation and the resultant retardation of photo-stimulated testicular recrudescence. A comparison of the observations between NLD and LD 18:6 also reveals the ability of long photoperiod-induced activation of the HHG axis to attenuate the inhibitory influences of DXM and CORT.

Another significant observation is the marked hypertrophy and hyperplasia of CORT treated birds under both NLD and LD 18:6.Apparently, CORT seems to have a favourable influence on Leydig cell functions. Pertinently, stimulatory influence of CORT on gonadal functions of sexually inactive japanese quail has also been reported (Peczely,1985). Despite the fact that photo-gonadal stimulation and parallel adrenal- testes relationship have been reported for birds since quite some time, it is ironical that no studies involving photoperiod-adrenal interaction have been attempted. This precludes any detailed discussion of the topic, as the present study is only one of its type. Overall,it can be concluded from the present observations that

1. Photoperiod has positive influence on testicular recrudescence in the pigeons.
2. The adrenocortical hormone has stimulatory influences on Leydig cell functions.
3. Dexamethasone and corticosterone may have a common site of action for HHT activation and
4. Corticosterone may have definite phase relationship with other hormone like PRL in regulating seasonal reproductive activities in pigeons.