CHAPTER 3

TRANSIENT NEONATAL HYPOTHYROIDISM AND PINEALECTOMY DELAYS TESTIS MATURATION AND ACCESSORY ORGAN GROWTH, BUT INCREASES ULTIMATE GERM CELL POPULATION IN THE RAT

Hypothyroidism is a common disorder, the effects of which manifest on various systems and metabolic functions (De Visscher and Ingelbleek, 1980). Literature is replete with reports of varying effects of hypothyroidism at various age groups in animals and man, which have precluded the emergence of a unified concept. Some earlier studies reported slight retardatory influence on the growth of the testes and epididymis due to hypothyroidism in rats (Hammet, 1923; Del Rio *et al.*, 1979). Thyroidectomy in adult rats was shown to be ineffective on the functions of the male reproductive system (Vilchez-Martinez, 1973; Amin and El-Sheikh, 1977), though some others could see degenerative changes (Amin and El-Sheikh,1977). Similarly, serum gonadotropins and testosterone levels in hypothyroid adult rats have been reported to be decreased (Baksi, 1973; Bruni *et al.*, 1975; Amin and El-Sheikh, 1977) or unchanged (Weiss and Burns, 1988). However, hypothyroidism in immature rats have been known to affect the male reproductive system. Some studies showed severe inhibition of spermatogenesis and Leydig cell development by thyroidectomy in immature rats (Chowdhry *et al.*, 1984; Weiss and Burns, 1988). However, Meisami (1984) reported for the first time markedly enlarged testis in adult male rats recovering from early hypothyroidism. This unique observation when further investigated was

shown to have a sensitive period during the first two weeks, hypothyroidism at which time could progressively result in testis weight nearly twice the normal adult size by 210 days of age (Meisami *et al.*, 1992).

Though the above hypertrophic response of testis in hypothyroid rats was demonstrated in the Long-Evans and Sprague-Dawley strains, similar experimental protocol employed on the Charles foster strain rats failed to reproduce the effect. In that study testis growth was found to be retarded significantly till 60 days though there was a tremendous growth between 60 and 90 days to reach almost 70% of the control testis weight (chapter 1). However, neonatal Px in this strain could result in 20% heavier testis size by 90 days compared to age matched controls (chapter 2). Further, the enlarged testis of Px animals was seen to contain greater number of germ cells and even increased interstitium. Earlier studies on pineal had reported insignificant effect of Px or melatonin administration in adult rats (Motta *et al.*, 1967; Pits and Maya, 1969; Reiter, 1973; 1980; Reiter *et al.*, 1985). Similar studies of Px and melatonin administration in immature rats showed earlier pubescence and slightly increased testis weight due to neonatal Px and, a delaying of pubescence when melatonin was administered only between 20 and 40 days of age. The overall conclusion had been, an inconsequential effect of pineal on the male reproductive functions in the rat. But in our study, there was significant hyperplastic response of testis and accessory organs after adolescence in neonatally pinealectomised rats.

Some reports have also indicated subtle pineal-thyroid interrelationship (Nir and Hirschmann, 1978; Brammer *et al.*, 1979; Virend, 1983; Heldmaier and Lynch, 1986; Lewinski, 1986; Ruzsas and Mess, 1987). It is apparent by now, that apart from having some subtle inter-relationship between them, these two endocrines do have some definite influence on the male reproductive system at defined critical phases during the postnatal development. This and the previous observations on the effects of transient neonatal hypothyroidism or Px on the male

reproductive system (chapters 1 and 2), provided impetus to study the possible influence of a combination of these two experimental paradigms.

RESULTS

I. MORPHOMETRIC OBSERVATIONS

BODY WEIGHT (Table 3.1; Fig. 1 & 2)

The body weight was significantly less in HPOT + Px rats at 35 day compared to that of the control animals. The body weight showed continuous increase from 35 to 90 days in control animals except for a slight decline between 35 and 45 days. The HPOT + Px rats showed a similar pattern of body weight increase like the control, though the body weight gain was always lesser than that of control rats throughout. At 90 days, the final weight was significantly less than the control rats in HPOT + Px animals. Also, when compared with the final body weight at 90 days with those of HPOT and Px groups of rats, they had the least body weight. On a percentage basis, the control animals showed maximum weight gain between 45 and 60 days. During the same period HPOT + Px animals also showed highest weight gain and it was more pronounced in HPOT + Px animals. Similar pattern of changes were depicted by the growth rate of both the groups of rats at all stages.

ORGAN WEIGHTS

Testes (Table 3.2 a, b; Fig. 3 a, b & 4)

The HPOT + Px animals had significantly lesser testes weight compared to the controls at 35 days. In the control rats, the testes weight increased continuously and steadily between 35 and 90 days, whereas the HPOT + Px animals showed very slight increase in testes weight between 35 and 60 days with, an actual decrement between 35 and 45 days. The testes weight increased significantly between 60 and 90 days. Both the control and HPOT + Px animals had comparable final testes weight at 90 days. In terms of relative weights, though there was no significant difference at 35 days in both control and HPOT + Px animals, it was slightly higher in control

Table 3.1 Chronological alterations in Body Weight (gm), Percentage Difference and Per Day Growth Rate in intact and hypothyroid rats subjected to pinealectomy (HPOT + Px)

Treatment	BODY WEIGHT				PERCENTAGE DIFFERENCE				PER DAY GROWTH RATE			
	Age in Days				Age in Days				Age in Days			
	35	45	60	90	35-45	45-60	60-90	35-90	0-35	35-45	45-60	60-90
Control	58.20 ± 6.36@	53.20 ± 3.07	89.70 ± 2.71	120.34 ± 9.77	- 8.59	+ 68.61	+ 34.16	+ 106.77	1.66 ± 0.08	-	2.43 ± 0.13	1.02 ± 0.09
HPOT + Px	42.60 ± 5.97^{c}	34.68 ± 4.49^{d}	71.18 ± 9.61^{c}	96.38 ± 8.77^{c}	- 18.59	+ 105.24	+ 35.40	+ 126.24	1.22 ± 0.09^{a}	-	2.43 ± 0.12^{ns}	0.84 ± 0.06^{c}

@ Values expressed as Mean ± SD of five experiments

^{a} $p < 0.05$; ^{c} $p < 0.01$; ^{d} $p < 0.001$; ^{ns} Not Significant

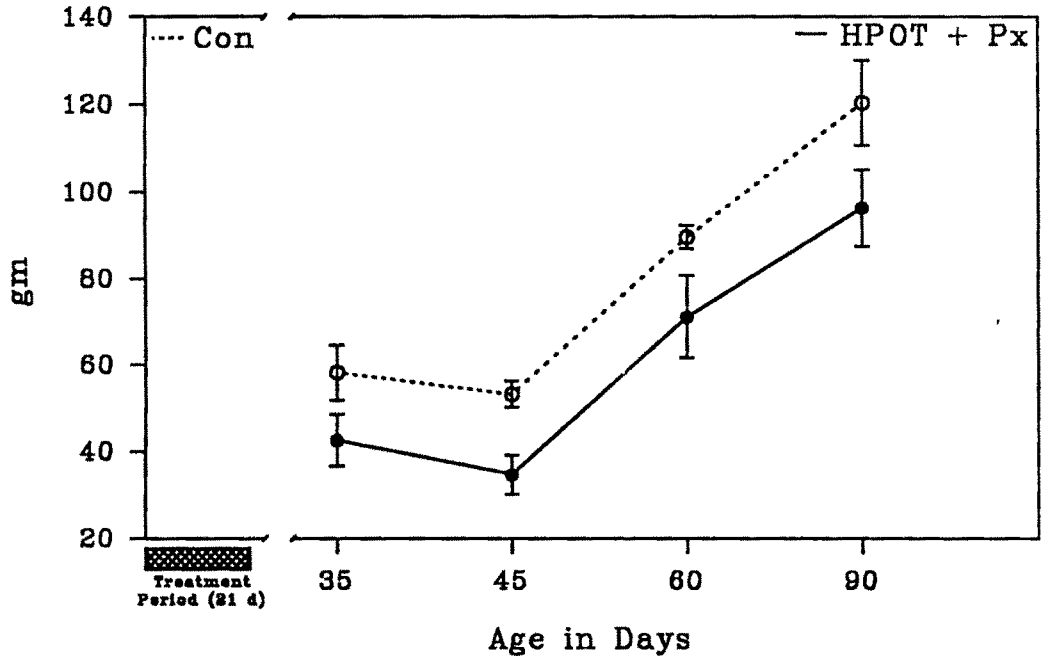


Fig. 1 Chronological alterations in body weight of hypothyroid rats subjected to pinealectomy (HPOT + Px)

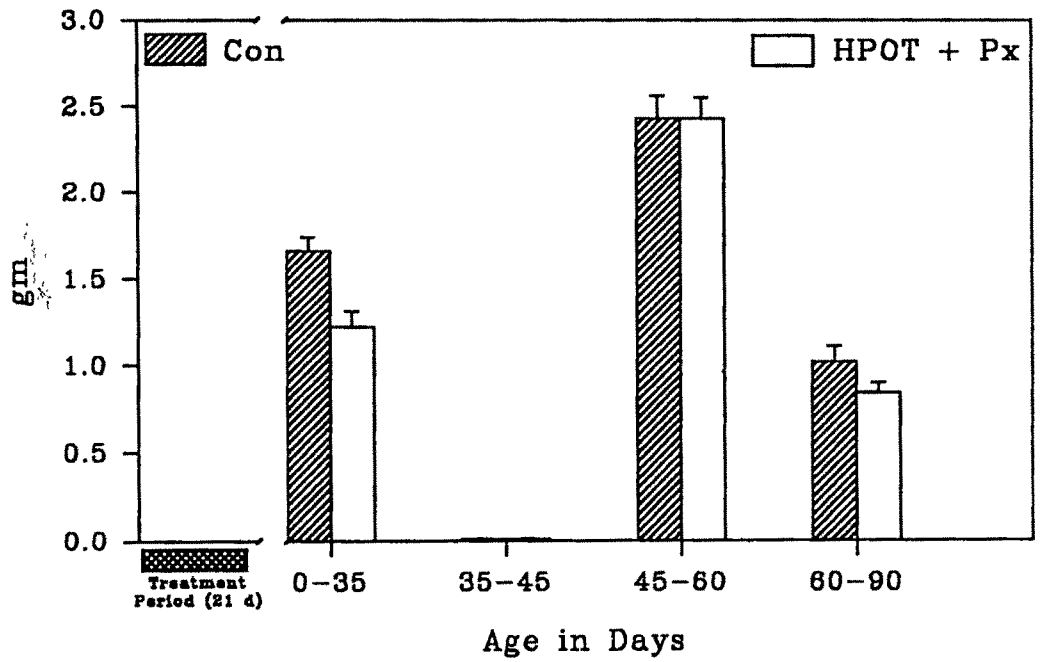


Fig. 2 Per day body growth in intact and HPOT + Px rats

Table 3.2 (a & b)Chronological alterations in Weight [Absolute (mg) and Relative (mg/100 mg)], Percentage Difference and Per Day Growth Rate of Testes and Epididymis in intact (Con) and hypothyroid rats subjected to pinealectomy (HPOT + Px)

Table a

ORGAN	TREATMENT	ABSOLUTE WEIGHT				RELATIVE WEIGHT			
		Age in Days				Age in Days			
		35	45	60	90	35	45	60	90
TESTES	Con	477.08@ ± 41.78	727.28 ± 36.08	1397.00 ± 80.27	2438.18 ± 57.88	0.83 ± 0.14	1.37 ± 0.12	1.56 ± 0.07	2.03 ± 0.02
	HPOT + Px	284.54^{d} ± 17.81	186.00^{d} ± 7.58	361.24^{d} ± 23.47	2372.80^{ns} ± 62.74	0.67^{b} ± 0.04	0.54^{d} ± 0.03	0.51^{d} ± 0.04	2.47^{d} ± 0.12
EPIDIDYMIS	Con	122.86 ± 8.37	64.28 ± 2.27	394.40 ± 10.24	503.84 ± 15.43	0.22 ± 0.04	0.12 ± 0.01	0.44 ± 0.02	0.42 ± 0 02
	HPOT + Px	125.00^{ns} ± 7.39	39.00^{d} ± 2.15	87.66^{d} ± 4.37	503.46^{ns} ± 14.23	0.29^{c} ± 0.02	0.11^{ns} ± 0.01	0.12^{d} ± 0.009	0.52^{d} ± 0.03

Table b

ORGAN	TREATMENT	PERCENTAGE DIFFERENCE				PER DAY GROWTH RATE			
		Age in Days				Age in Days			
		35-45	45-60	60-90	35-90	0-35	35-45	45-60	60-90
TESTES	Con	+ 52.46	+ 92.06	+ 74.53	+ 411.06	13.63 ± 2.32	25.03 ± 3.42	44.60 ± 4.05	34.70 ± 4.95
	HPOT + Px	- 34.63	+ 94.22	+ 556.85	+ 733.91	8.13^{c} ± 0.81	-	11.68^{d} ± 0.95	67.05^{d} ± 3 50
EPIDIDYMIS	Con	- 47.68	+ 513.56	+ 27.75	+ 310.09	3.51 ± 0.32	-	22.06 ± 6.45	3.65 ± 0 24
	HPOT + Px	- 68.80	+ 124.77	+ 474.33	+ 302.77	3.57^{ns} ± 0.29	-	3.24^{d} ± 0.34	13.86^{d} ± 1.05

@ Values expressed as Mean ± SD of five experiments; ^{b} $p < 0.025$; ^{c} $p < 0.01$; ^{d} $p < 0.001$; ^{ns} Not Significant

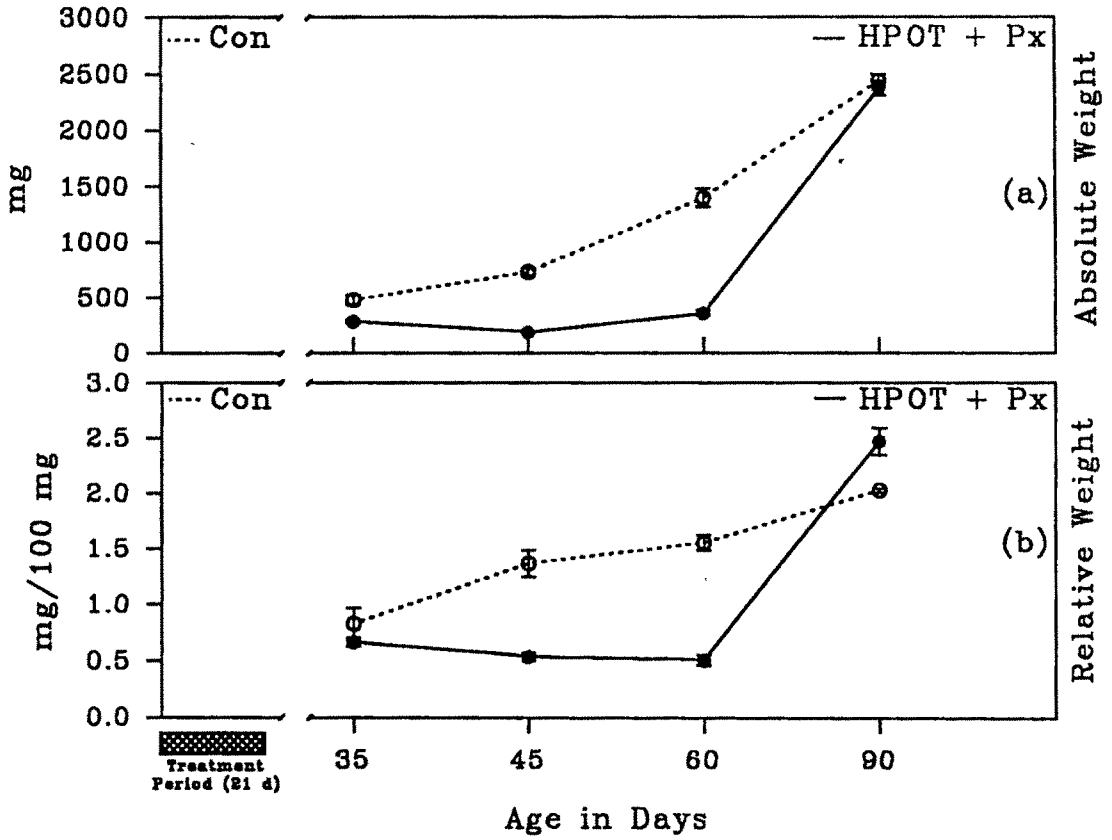


Fig. 3 (a&b) Chronological alterations in weights of testes in hypothyroid rats subjected to pinealectomy (HPOT + Px)

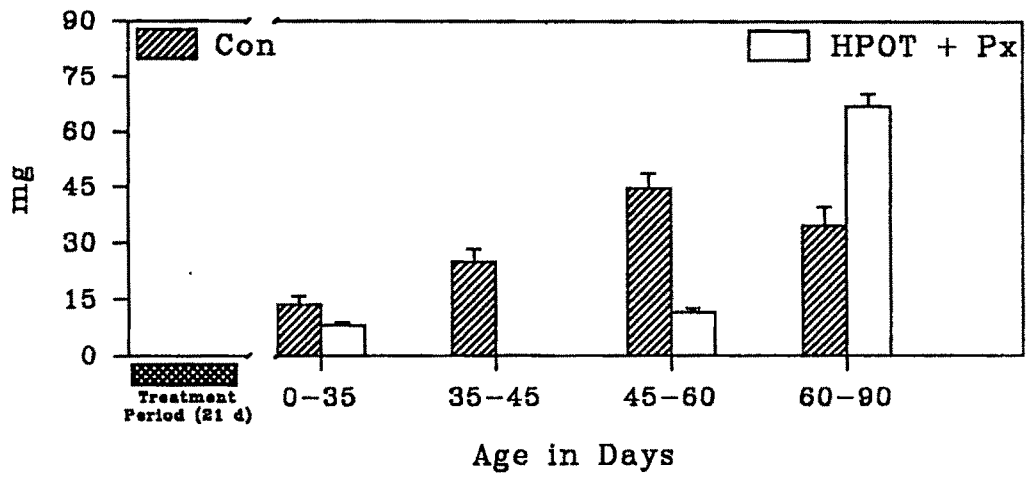


Fig. 4 Rate of growth of testes in intact and HPOT + Px rats

rats. Similar to the absolute weight, the relative weight of testes in the control animals depicted continuous increase between 35 and 90 days. The HPOT + Px animals showed decreased relative weight between 35 and 45 days and again a slight decrease between 45 and 60 days; however, the relative weight increased significantly between 60 and 90 days to attain the maximal weight comparable to the control rats. The HPOT + Px animals showed maximum growth between 60 and 90 days while in the control animals, it was between 45 and 60 days. Similar pattern of change was shown on a percentage basis by the testes in both the groups of animals. Among other experimental groups (i.e., Px and HPOT), HPOT + Px animals had the maximum testes weight at 90 days.

Epididymis (Table 3.2 a, b; Fig. 5 a, b & 6)
The weight of epididymis was similar in both control and HPOT + Px animals at 35 days though slightly low in the control group. At 45 days, both control and HPOT + Px animals showed a significant decrease in epididymis weight compared to 35 days. The decrease was more pronounced in HPOT + Px animals. Between 45 and 90 days the weight of the epididymis increased continuously and significantly in both control and HPOT + Px animals. Whereas the maximal growth spurt occurred between 45 and 60 days in control animals, the same occurred between 60 and 90 days in HPOT + Px animals. The ultimate absolute weight of epididymis at 90 days was similar in both the control and HPOT + Px animals. Relative weight of epididymis in both the groups of rat paralleled the changes in absolute weight at all stages. The final weight of epididymis at 90 days was intermediate both in control and HPOT + Px animals when compared with that of the Px and HPOT animals. The Px animals showed the highest and the HPOT rats showed the lowest epididymis weight at 90 days.

Seminal Vesicle (Table 3.3 a, b; Fig. 7 a, b & 8)
The absolute weight of seminal vesicle at 35 days was significantly less in HPOT + Px animals compared to the control rats. The weight of seminal vesicle showed a decrease at 45 days in both control and HPOT + Px animals. Between 45 and 90 days the weight of seminal vesicle

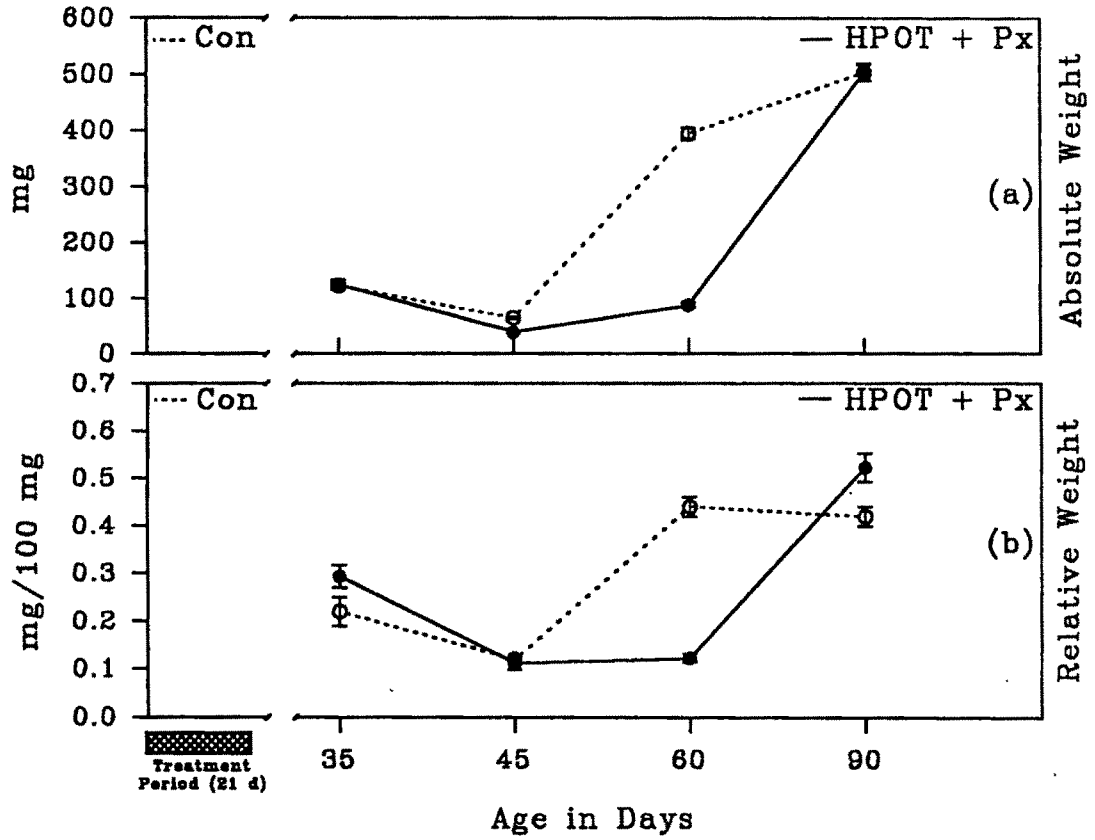


Fig. 5 (a&b) Chronological alterations in weights of epididymis in hypothyroid rats subjected to pinealectomy (HPOT + Px)

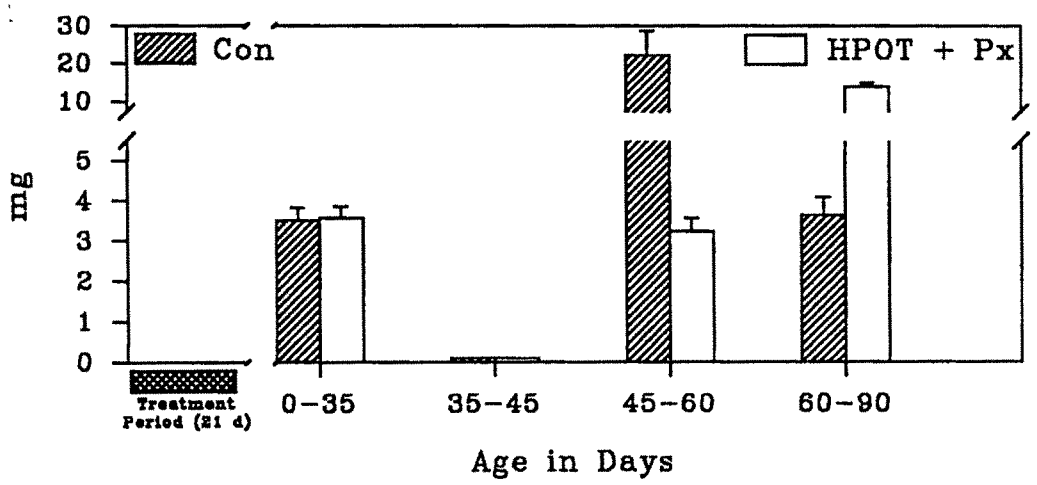


Fig. 6 Rate of growth of epididymis in intact and HPOT + Px rats

Table 3.3 (a & b)Chronological alterations in Weight [Absolute (mg) and Relative (mg/100 mg)], Percentage Difference and Per Day Growth Rate of Seminal Vesicle and Prostate Gland in intact (Con) and hypothyroid rats subjected to pinealectomy (HPOT + Px)

Table a

ORGAN	TREATMENT	ABSOLUTE WEIGHT				RELATIVE WEIGHT			
		Age in Days				Age in Days			
		35	45	60	90	35	45	60	90
SEMINAL VESICLE	Con	25.07@ ± 4.71	19.00 ± 3.70	66.94 ± 8.32	423.77 ± 13.99	0.043 ± 0.007	0.036 ± 0.002	0.074 ± 0.005	0.352 ± 0.009
	HPOT + Px	13.27^{d} ± 1.71	7.40^{d} ± 0.62	26.39^{d} ± 2.03	464.09^{c} ± 14.44	0.031^{c} ± 0.002	0.021^{d} ± 0.002	0.037^{d} ± 0.002	0.480^{d} ± 0.033
PROSTATE GLAND	Con	24.46 ± 3.61	15.13 ± 1.53	81.29 ± 10.99	154.35 ± 14.09	0.042 ± 0.004	0.028 ± 0.003	0.090 ± 0.009	0.128 ± 0.003
	HPOT + Px	22.58^{ns} ± 3.12	14.29^{ns} ± 2.96	24.90^{d} ± 4.13	196.09^{d} ± 11.93	0.053^{c} ± 0.008	0.041^{d} ± 0.004	0.035^{d} ± 0.003	0.204^{d} ± 0.014

Table b

ORGAN	TREATMENT	PERCENTAGE DIFFERENCE				PER DAY GROWTH RATE			
		Age in Days				Age in Days			
		35-45	45-60	60-90	35-90	0-35	35-45	45-60	60-90
SEMINAL VESICLE	Con	- 24.21	+ 252.32	+ 533.06	+ 1590.35	0.72 ± 0.06	-	3.19 ± 0.28	11.89 ± 1.05
	HPOT + Px	- 44.24	+ 252.62	+ 1658.58	+ 3397.28	0.38^{d} ± 0.05	-	1.27^{d} ± 0.29	14.59^{b} ± 1.90
PROSTATE GLAND	Con	- 37.94	+ 435.51	+ 89.87	+ 531.03	0.69 ± 0.09	-	4.41 ± 0.95	2.43 ± 0.89
	HPOT + Px	- 36.71	+ 74.25	+ 687.51	+ 768.42	0.65^{ns} ± 0.10	-	0.71^{d} ± 0.10	5.71^{d} ± 0.99

@ Values expressed as Mean ± SD of five experiments; ^{b} $p < 0.025$; ^{c} $p < 0.01$; ^{d} $p < 0.001$; ^{ns} Not Significant

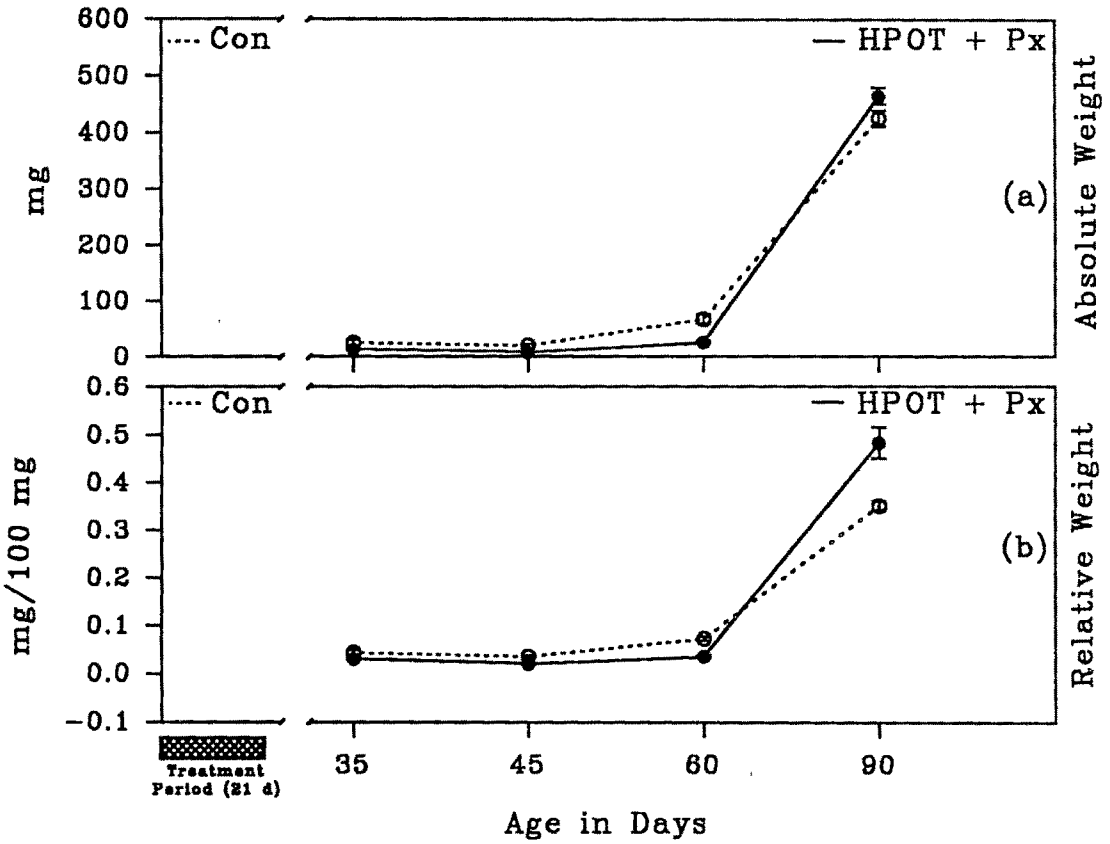


Fig. 7 (a&b) Chronological alterations in weights of seminal vesicle in hypothyroid rats subjected to pinealectomy (HPOT + Px)

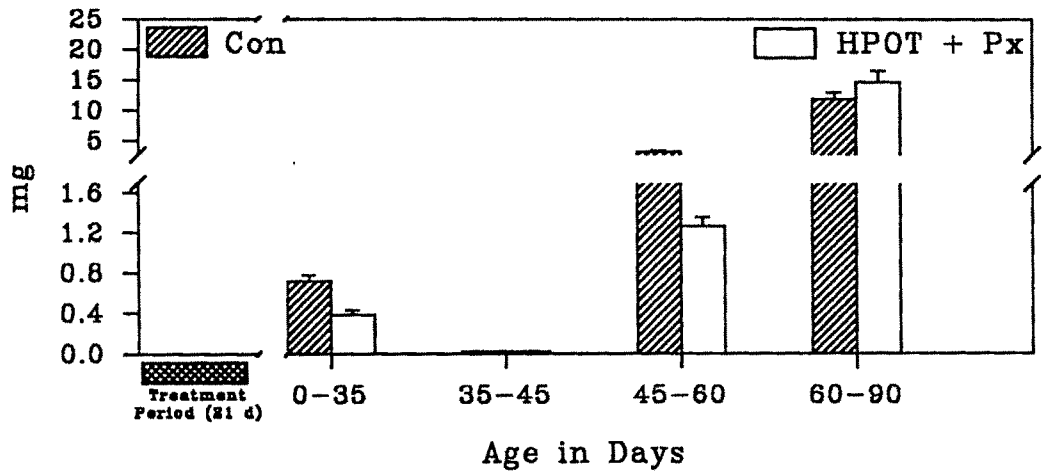


Fig. 8 Rate of growth of seminal vesicle in intact and HPOT + Px rats

increased continuously and significantly in both control and HPOT + Px animals. Both the groups depicted the maximum growth spurt between 60 and 90 days but was more pronounced in HPOT + Px animals. At 90 days, the final weight of the epididymis was identical but was slightly more in the HPOT + Px animals. The changes in the relative weight of seminal vesicle in both the groups at all the stages reflected the changes in the absolute weight. Both the control and HPOT + Px animals had intermediate final epididymis weight at 90 days when compared with the final weights of Px and HPOT animals. There was similarity between the pattern of changes depicted by the testes and that of the epididymis among all the groups at 90 days.

Prostate Gland (Table 3.3 a, b; Fig. 9 a, b & 10)

The weight of prostate gland at 35 days was more or less similar in control and HPOT + Px animals, though it was low in the HPOT + Px group. At 45 days, both the control and HPOT + Px animals showed a decrement in the prostate weight. In both control and HPOT + Px animals, the weight of prostate increased continuously between 45 and 90 days with a maximal growth rate between 45 and 60 days in the control and between 60 and 90 days in HPOT + Px animals. The final weight of prostate at 90 days was significantly high in the HPOT + Px animals compared to the control rats. The relative weight of the prostate in both the groups of animals at all the stages reflected the changes in absolute weight. Among all other experimental groups the ultimate weight of the prostate at 90 days was maximal in HPOT + Px and Px animals and minimal in HPOT animals. The control animals showed an intermediate final prostate weight compared to HPOT + Px and Px and HPOT animals.

Thyroid Gland (Table 3.4; Fig. 11 a & b)

The thyroid gland weighed almost double than that of the control in HPOT + Px rats at 35 days. At 45 days, there was significant decrement in the weight of thyroid in both the groups In the control animals there was continuous and steady increase in thyroid weight between 45 and 90 days, while in the HPOT + Px group of rats, the weight of the thyroid increased at 60 days and

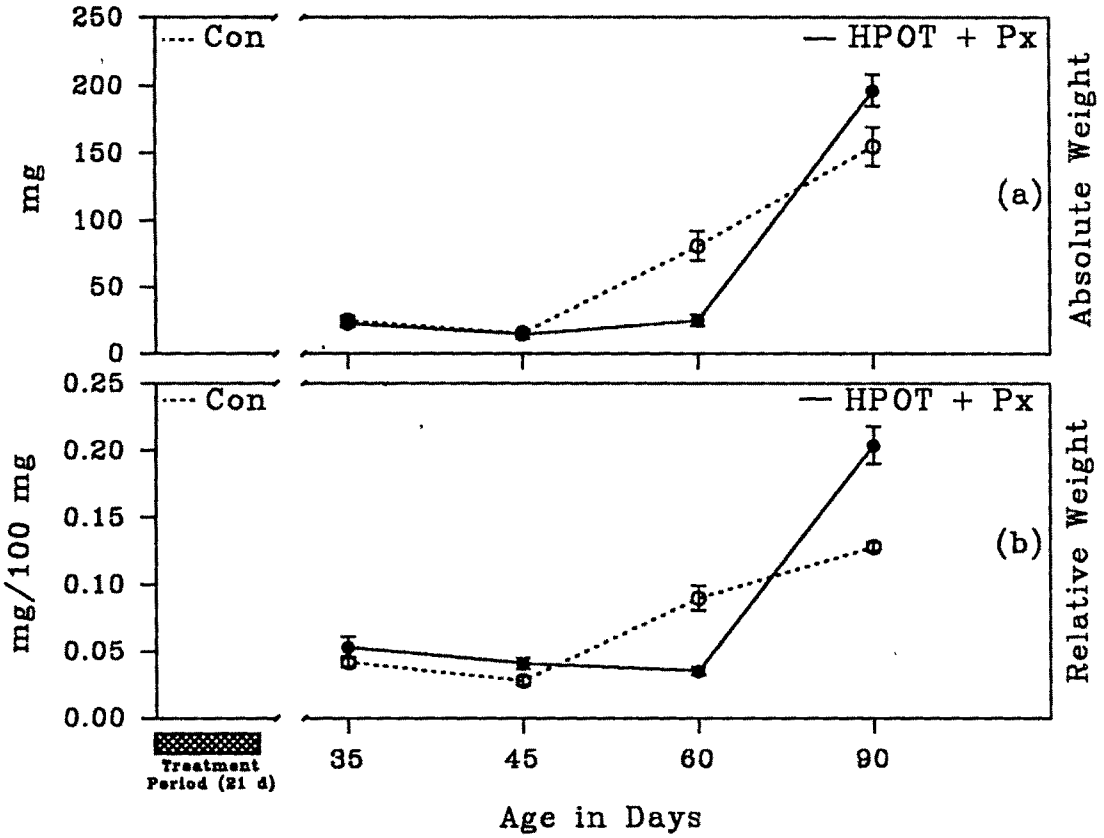


Fig. 9 (a&b) Chronological alterations in weights of prostate gland in hypothyroid rats subjected to pinealectomy (HPOT + Px)

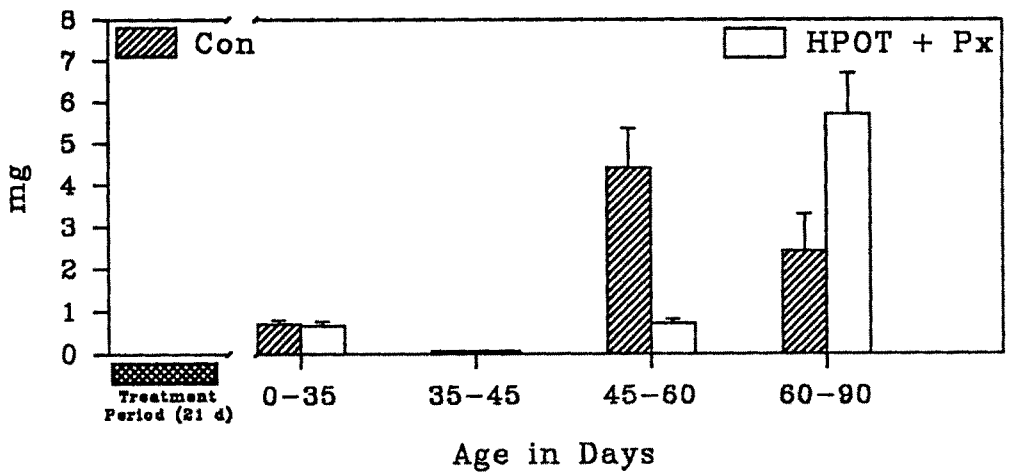


Fig. 10 Rate of growth of prostate gland in intact and HPOT + Px rats

Table 3.4 Chronological alterations in Weight [Absolute (mg) and Relative (mg/100mg)] and Percentage Difference of Thyroid Gland in intact and hypothyroid rats subjected to pinealectomy (HPOT + Px)

Treatment	ABSOLUTE WEIGHT				RELATIVE WEIGHT				PERCENTAGE DIFFERENCE			
	Age in Days				Age in Days				Age in Days			
	35	45	60	90	35	45	60	90	35-45	45-60	60-90	35-90
Control	6.00 ± 0.71@	3.52 ± 0.55	6.62 ± 0.44	8.10 ± 0.74	0.010 ± 0.002	0.006 ± 0.001	0.007 ± 0.0006	0.007 ± 0.0005	- 41.33	+ 88.07	+ 22.36	+ 35.009
HPOT + Px	12.76 ± 1.89^{d}	6.72 ± 0.56^{d}	11.68 ± 1.54^{d}	8.36 ± 1.16^{ns}	0.030 ± 0.005^{d}	0.019 ± 0.002^{d}	0.016 ± 0.002^{d}	0.009 ± 0.001^{b}	+ 47.34	+ 73.81	- 28.42	- 34.48

@ Values expressed as Mean ± SD of five experiments

^{b} $p < 0.025$; ^{d} $p < 0.001$; ^{ns} Not Significant

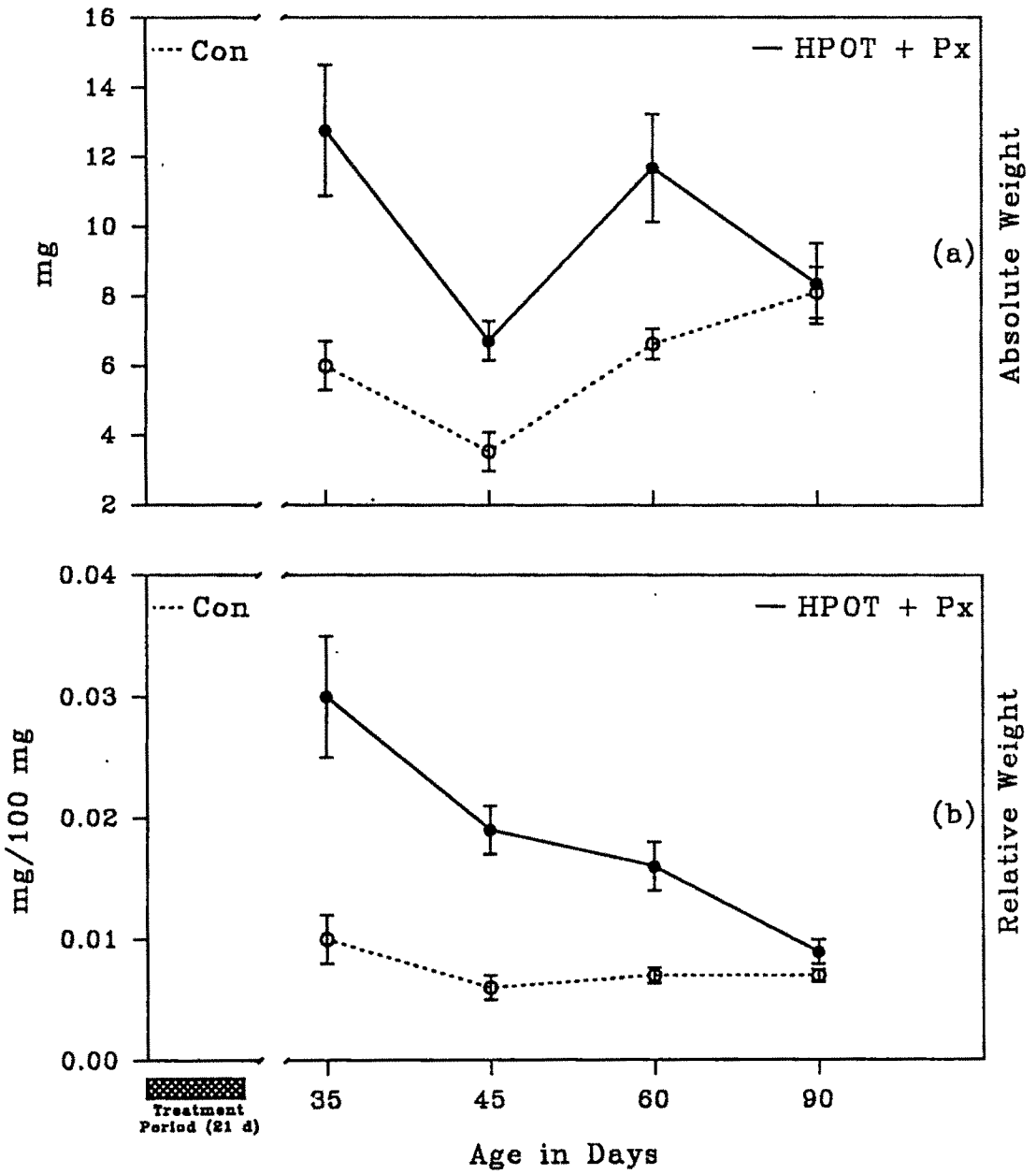


Fig. 11 (a&b) Chronological alterations in weights of thyroid gland inpothyroid rats subjected to pinealectomy (HPOT + Px)

then decreased to attain a weight similar to that of control animals at 90 days. Both the control and HPOT + Px rats showed the maximal weight gain of thyroid between 45 and 60 days. The relative weight of thyroid in both the groups was maximal at 35 days and was more pronounced in the HPOT + Px animals. The relative weight of thyroid at 90 days was minimal in both the groups, though the HPOT + Px animals had greater relative weight than the control group of rat.

II. HISTOLOGICAL OBSERVATIONS

STRUCTURE OF TESTIS (Table 3.5; Plates I & II)

35 Day Old

Control: The tubules were small with an average diameter 90.47 μm with mostly spermatogonial cells and primary spermatocytes in the zygotene stage. Some tubules also showed few pachytene spermatocytes . Lumenation of the tubules was evident with many tubules showing degenerating germ cells in the lumen. Interstitial cells were mostly small and inactive though occasionally at regions, few active hypertrophied ones also could be seen.

HPOT + Px: The tubules were smaller in size like in the HPOT animals with an average diameter of 71.43 μm. The germ cell population appeared intermediate between those of HPOT and Px. although still less than the controls. Like in the Px animals, degenerating cells were less evident. Most of the primary spermatocytes were in the zygotene stage. The interstitium appeared to be moderately developed.

45 Day Old

Control: The tubules were enlarged with an average diameter of 114.28 μm. Spermatogenesis was more advanced and was marked by the appearance of post-zygotene primary spermatocytes and even secondary spermatocytes and round spermatids. There was also evidence of spermatogonial proliferation. The interstitium was well developed.

Table 3.5 Chronological alterations in the Diameter (in μm) of Seminiferous Tubule and Epididymis (Caput and Cauda) in intact and hypothyroid rats subjected to pinealectomy (HPOT + Px)

Treatment	SEMINIFEROUS TUBULE				EPIDIDYMIS							
					CAPUT				CAUDA			
	Age in Days				Age in Days				Age in Days			
	35	45	60	90	35	45	60	90	35	45	60	90
Control	90.47 ± 5.86@	114.28 ± 7.98	162.86 ± 12 16	187.53 ± 12 84	23.24 ± 1.09	26.96 ± 1.21	31.92 ± 2.82	49.67 ± 2.72	11.48 ± 1 16	13.94 ± 0.96	20.24 ± 1.85	21.57 ± 2.02
HPOT + Px	71.43 ± 3.12^{d}	104.76 ± 5.46^{c}	117.24 ± 8.26^{d}	144.23 ± 12.33^{d}	28.31 ± 2.01^{b}	31.81 ± 2.87^{b}	33.43 ± 1.99^{ns}	33 43 ± 3.10^{d}	17.95 ± 1.48^{d}	19.05 ± 1.91^{d}	17.86 ± 1.86^{b}	17.71 ± 1.98^{c}

@ Values expressed as Mean ± SD of five experiments

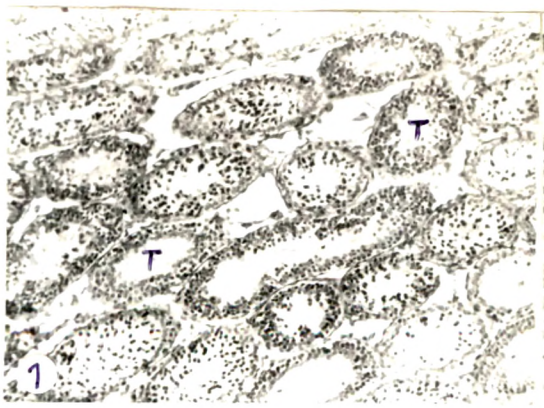
^{b} $p < 0.025$; ^{c} $p < 0.01$; ^{d} $p < 0.001$; ^{ns} Not Significant

PLATE I

Figures 1-6: Photomicrographs of sections of testis of 35 and 45 day old transient hypothyroid rats subjected to neonatal pinealectomy (HPOT + Px).

Figures 1 (100 x) and 4 (200 x): Sections of testis of 35 day old rat showing smaller tubules (T) with germ cells up to spermatocytes (Sc) and less degeneration of cells (arrow).

Figures 2,3 (100 x) and 5,6 (200 x): Sections of testis of 45 day old rats showing slightly enlarged tubules (T) and germ cells up to spermatocytes (Sc). Note degenerative changes (arrow) of spermatocytes in some of the tubules. Interstitium (I) is well developed.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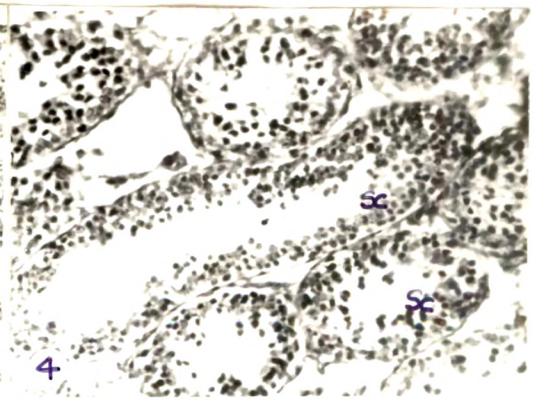
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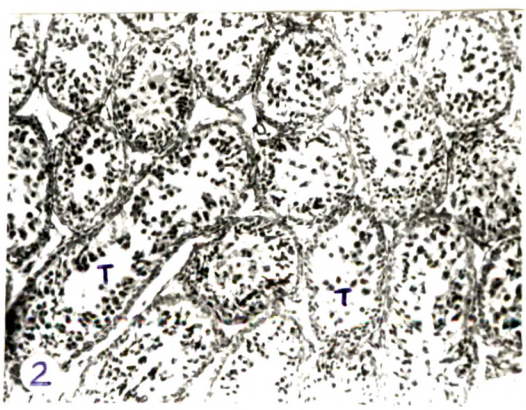
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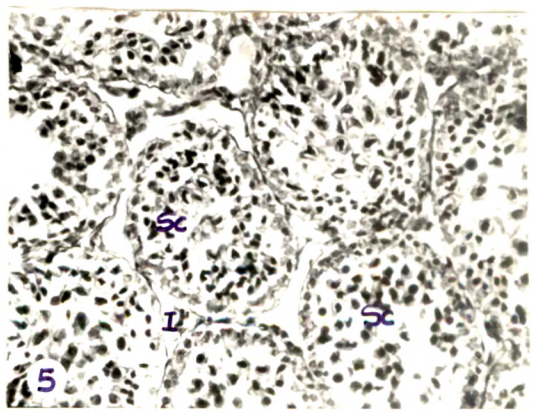
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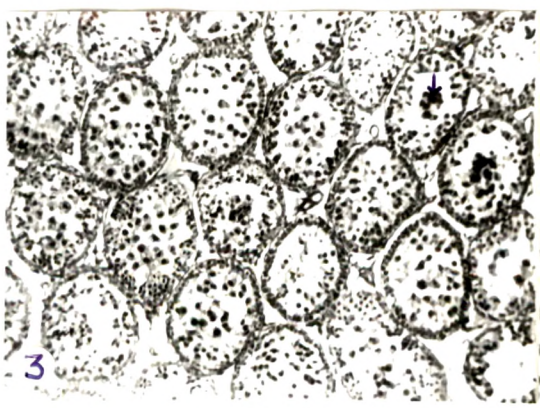
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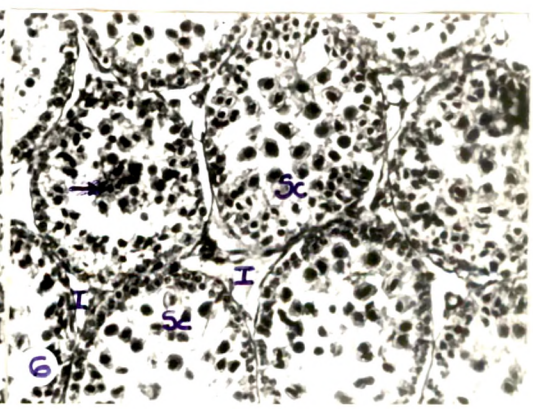
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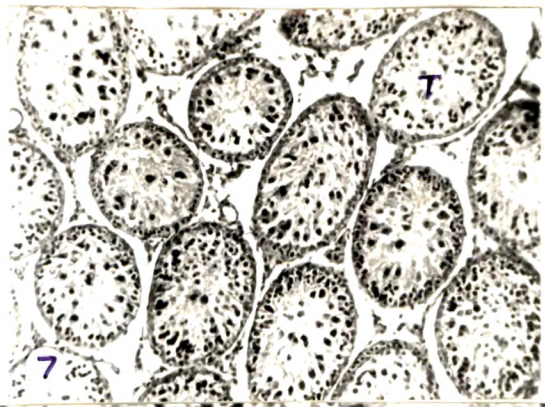
PLATE II

Figures 7-13: Photomicrographs of sections of testis of 60 and 90 day old HPOT + Px rats.

Figures 7 (100 x) and 8,9 (200 x): Sections of testis of 60 day old rat showing larger tubules (T) than HPOT and spermatogenesis had progressed up to round spermatids (RSt). Interstitium (I) is well developed.

Figures 10,11 (100 x) and 12,13 (200 x) : Sections of testis of 90 day old rat showing enlarged compact tubules. Most of the tubules show elongating spermatids (ESt) and a few show spermatozoa (Sz). Interstitium (I) is prominent.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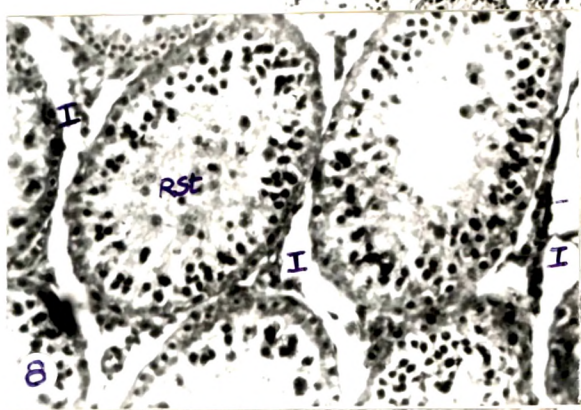
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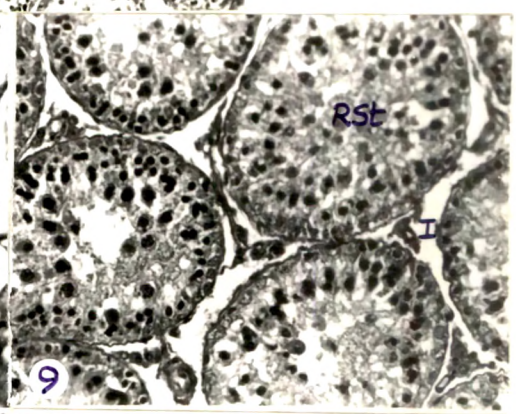
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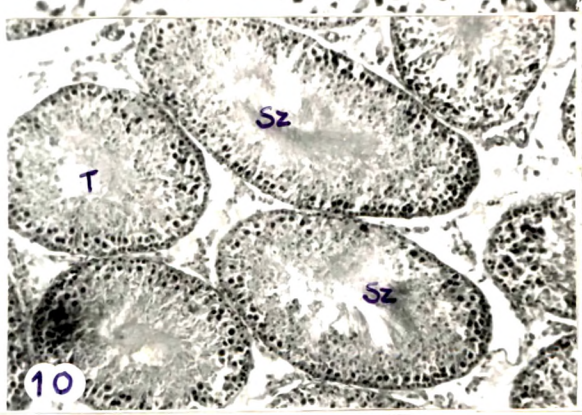
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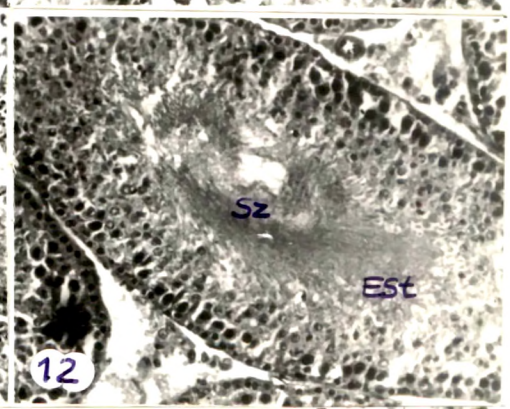
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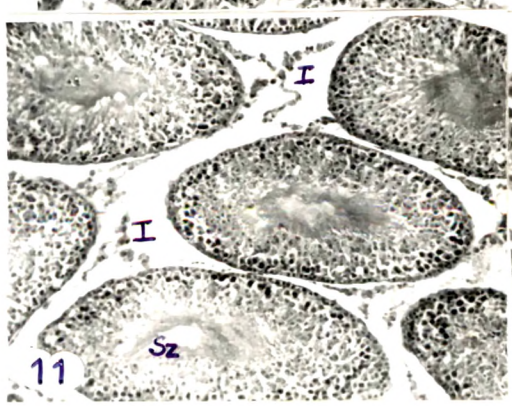
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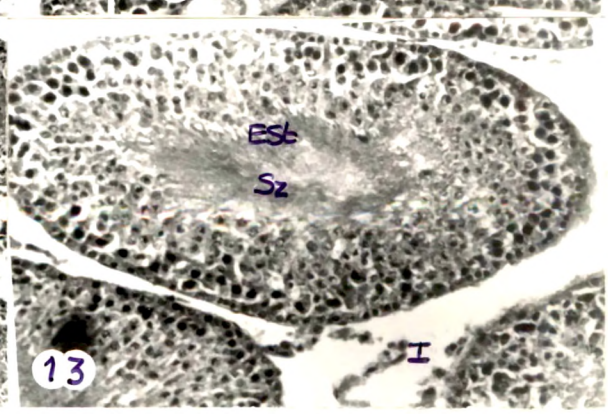
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HPOT + Px: The tubules were enlarged as compared to 35 days (104.76 um), and was less than the controls and Px animals but more than HPOT. The tubules were well formed and spermatogenesis appeared to progress up to primary spermatocytes. In many tubules some of the spermatocytes appeared to be undergoing degeneration. The interstitium was better developed.

60 Day Old

Control: The tubules increased in diameter further and attained a size of 162.86 μm in diameter. They were well formed and spermatogenesis was complete with many tubules showing spermatids and spermatozoa. The interstitium was well developed.

HPOT + Px: The tubular enlargement was nominal (117.24 μm) and was bigger in size compared to the HPOT animals but less than the control and Px animals. Tubules were well formed and spermatogenesis was re-established with many tubules showing round and elongating spermatids. The overall cell population appeared to be increased. The interstitium was well developed.

90 Day Old

Control: The tubules were further enlarged with a maximum diameter of 187.52 μm. Spermatogenesis was fully established in all the tubules and most of the tubules were having sperms. The interstitium appeared to be moderately developed.

HPOT + Px: The tubules showed enlargement and measured 144.33 μm, which was more like that of HPOT animals and less than that of control and Px animals. The tubules appeared compact and the overall germ cell population in them was more than HPOT. The most advanced germ cell types that could be seen were round spermatids and early elongating spermatids. Spermatid differentiation appeared to be affected though in some cases, differentiation towards spermatozoa was evident and, in some individuals, tubules even showed sperms. Overall there

seems to be variation from no spermatid differentiation to occurrence of spermatozoa. Interstitium appeared prominent and more in number.

STRUCTURE OF EPIDIDYMIS (Table 3.5; Plate III)

35 Day Old

Control: The tubules were lined by cuboidal to columnar epithelial cells and the cell height varied between 11.48 to 23.24 μm. In between the tubules fibrous connective tissue was evident. Degenerated germ cells flushed out from the testis could be seen in the lumen.

HPOT + Px: The tubules appeared compactly packed with hypertrophied epithelial cells whose diameter ranged between 17.95 to 28.33 μm. The lumen appeared narrow.

45 Day Old

Control: The tubules were well developed and compactly packed and the cell height ranged between 13.9 to 26.9 μm. The lumen was filled with round spermatids.

HPOT + Px: The tubules were large with greatly hypertrophied cells whose diameter ranged between 19.05 to 31.8 μm. The lumen of the tubules appeared narrow.

60 Day Old

Control: The epididymis appeared well developed with large compactly packed tubules with cell height ranging between 20.24 to 31.9 μm.

HPOT + Px: The tubules were well formed with large epithelial cells whose height varied between 17.8 to 33.4 μm, more like the control animals. The lumen was almost empty except for a few germ cells.

90 Day Old

Control: The well formed large tubules were lined by cuboidal to columnar epithelial cells. The cell height was maximum ranging between 21.6 to 49.7 μm. The lumen was filled with sperms.

PLATE III

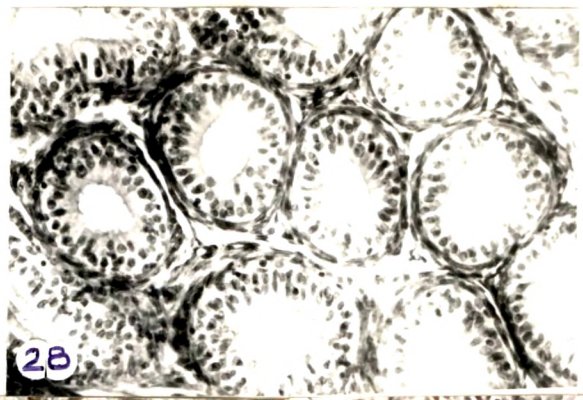
Figures 28-33: Photomicrographs of sections of epididymis of 35,45,60 and 90 day old HPOT + Px rats (200 x).

Figure 28: Section of epididymis of 35 day old rat showing small compactly packed tubules with hypertrophied epithelium.

Figures 29 and 30: Sections of epididymis of 45 day old rat showing slightly larger tubules with hypertrophied epithelial cells and narrow lumen. The lumen contains degenerating germ cells.

Figure 31: Section of epididymis of 60 day old rat showing well formed tubules lined by large epithelial cells. The lumen is with few degenerating cells.

Figures 32 and 33: Sections of epididymis of 90 day old rat showing large well formed tubules with prominent epithelium. The lumen contains mostly spermatids.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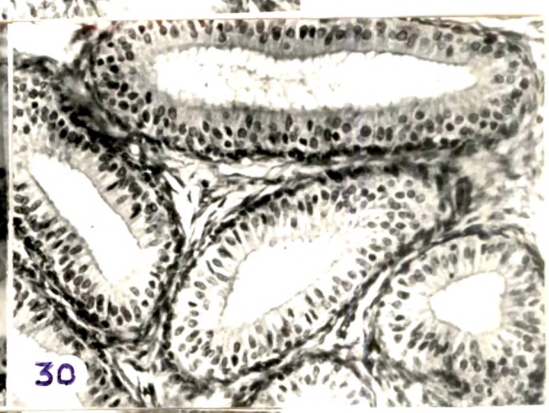
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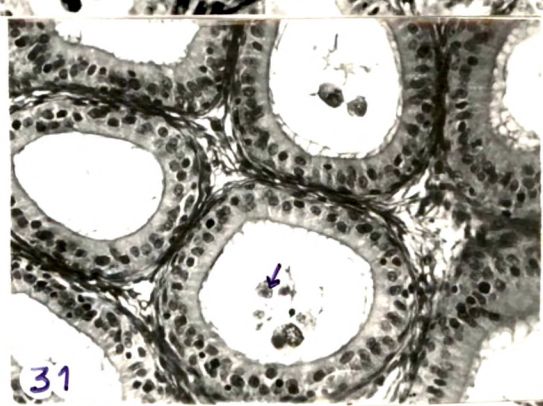
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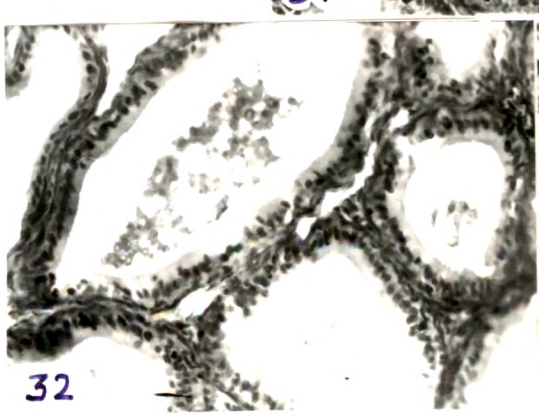
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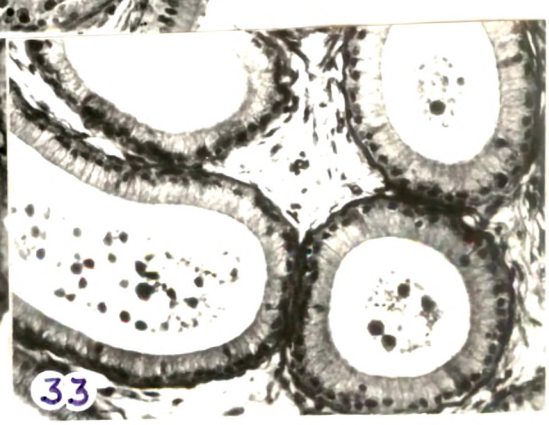
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HPOT + Px: The tubules were large and well formed with prominent epithelial cells whose height varied between 17.7 to 33.4 μm, similar to that of Px and HPOT animals and less than that of controls. The lumen contained germ cells.

STRUCTURE OF SEMINAL VESICLE (Plate IV)

35 Day Old

Control: The secretory epithelium was small and less convoluted with no secretory material.

HPOT + Px: The epithelium was well developed and convoluted with hypertrophied cells.

45 Day Old

Control: The secretory epithelium appeared better developed than at 35 days and was convoluted.

HPOT + Px: The epithelium was well developed with hypertrophied cells. Presence of very little amorphous secretory material was evident in the lumen.

60 Day Old

Control: The epithelium was well developed and convoluted. The cells were from cuboidal to columnar and the lumen contained secretory material.

HPOT + Px: The epithelium was prominent and convoluted and lined by hypertrophied cells. The lumen was filled with abundant secretory material

90 Day Old

Control: The secretory epithelium was very well developed and highly convoluted. It was lined by tall columnar cells and the narrow lumen was filled with secretory material.

HPOT + Px: The epithelium was prominent and convoluted and was lined by normal to slightly hypertrophied cells. There was quantitatively abundant secretory material.

PLATE IV

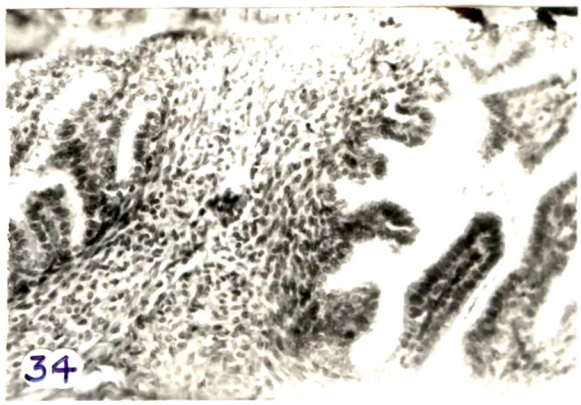
Figures 34-38: Photomicrographs of sections of seminal vesicle of 35,45,60 and 90 day old HPOT +Px rats (200 x).

Figure 34: Section of seminal vesicle of 35 day old rat showing well developed convoluted secretory epithelium with slightly hypertrophied cells.

Figures 35 and 36: Sections of seminal vesicle of 45 day old rat showing well developed epithelium, more convoluted with hypertrophied cells. The lumen contains little secretory materials.

Figure 37: Section of seminal vesicle of 60 day old rat showing very prominent convoluted epithelium lined by hypertrophied cells, the lumen contain colloidal secretory material (S).

Figure 38: Section of seminal vesicle of 90 day old rat showing prominent convoluted epithelium lined by normal to slightly hypertrophied cells.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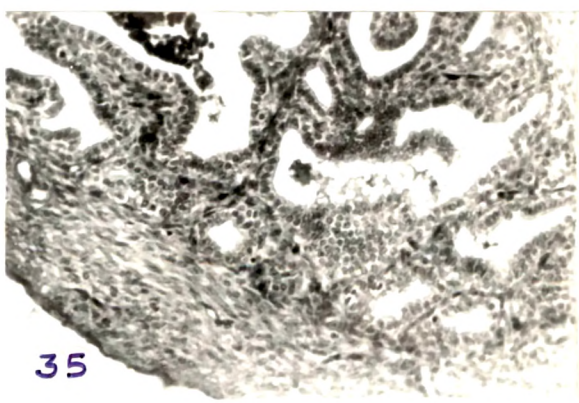
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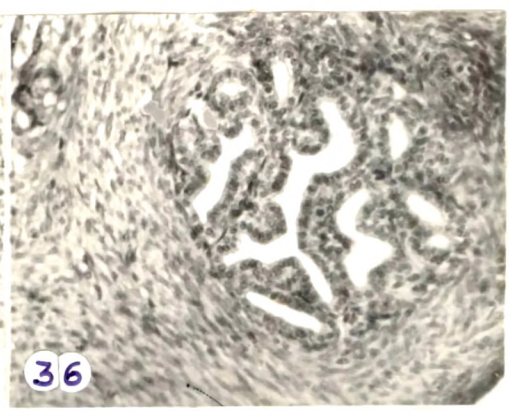
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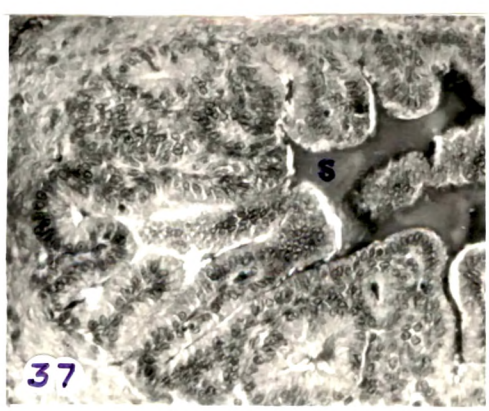
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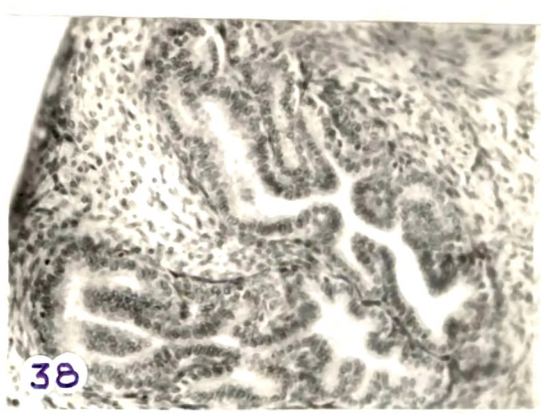
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STRUCTURE OF PROSTATE (Plate V)

35 Day Old

Control: The prostatic acini was less convoluted and lined by cuboidal to columnar cells. Some secretory material could be seen in the lumen.

HPOT + Px: The acini were lined by hypertrophied cells and were large cuboidal to columnar. The acini were slightly convoluted.

45 Day Old

Control: The acini were well developed and convoluted and lined by tall columnar epithelium. The epithelium also appeared pseudostratified.

HPOT + Px: The acini were well developed and lined by hypertrophied, large cuboidal to tall columnar epithelial cells. Amorphous secretory material was present in the lumen.

60 Day Old

Control: The acini were well developed and lined by tall columnar cells and with amorphous secretory material in the lumen.

HPOT + Px: The prostatic acini were large and convoluted. They were lined by cuboidal to columnar epithelial cells and the lumen contained abundant secretory material.

90 Day Old

Control: The acini were large, prominent and lined by tall columnar cells. The lumen was filled with amorphous secretion and some cells.

HPOT+Px: The prostatic structure appeared normal with large, prominent acini and with secretory material in the lumen.

STRUCTURE OF THYROID (Plate VI)

35 Day Old

Control: Thyroid appeared active with the follicles lined by large cuboidal cells. The lumen was narrow and contained very little colloid.

PLATE V

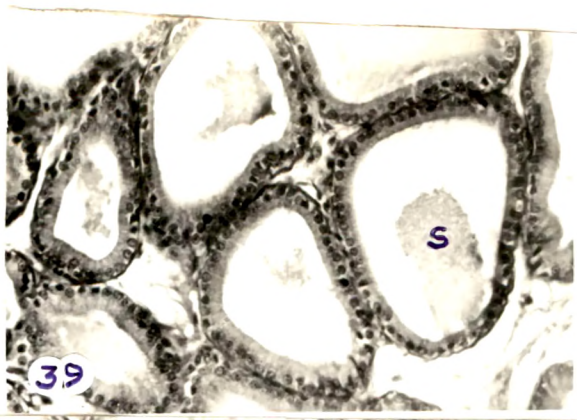
Figures 39-44: Photomicrographs of sections of prostate of 35,45,60 and 90 day old HPOT +Px rats (200 x).

Figure 39: Section of prostate of 35 day old rat showing secretory acini lined by cuboidal to columnar epithelial cells. Secretory material could be seen in the lumen.

Figures 40 and 41: Sections of prostate of 45 day old rat showing well developed acini lined by large cuboidal to tall columnar cells. Amorphous secretory material present in the lumen.

Figure 42: Section of prostate of 60 day old rat showing prominent convoluted acini lined by cuboidal to columnar epithelial cells. The lumen contains amorphous secretory material.

Figures 43 and 44: Sections of prostate of 90 day old rat showing large secretory acini lined by cuboidal to columnar epithelial cells. Secretory material present in the lumen.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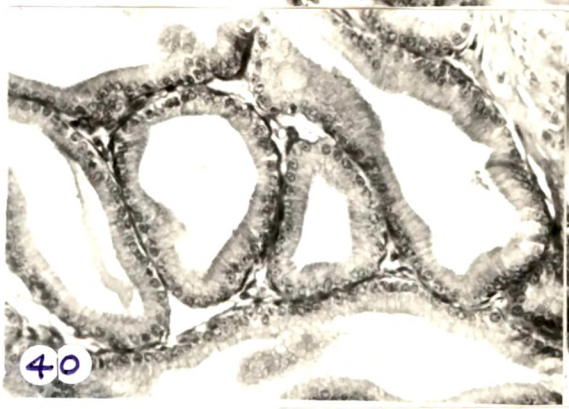
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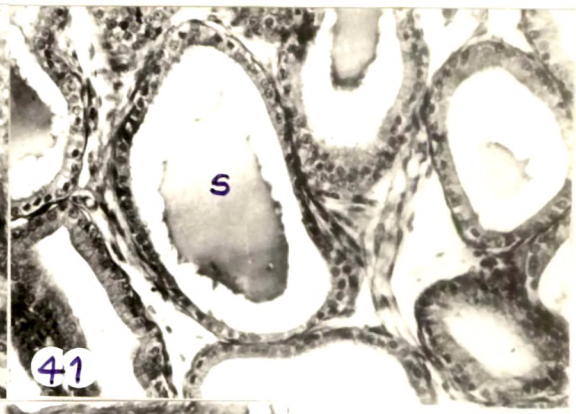
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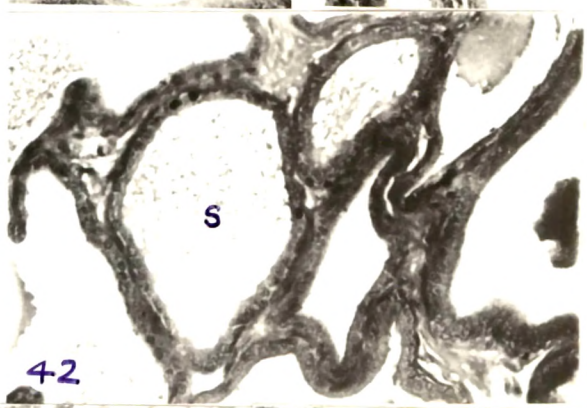
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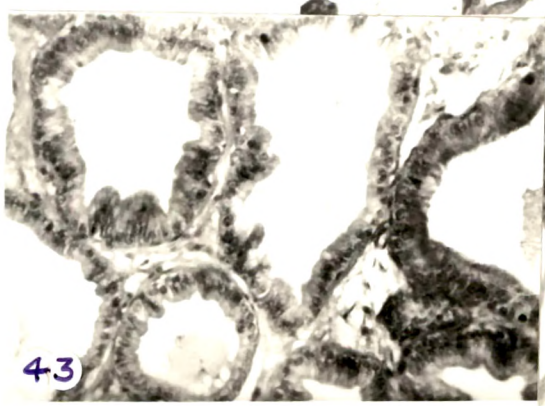
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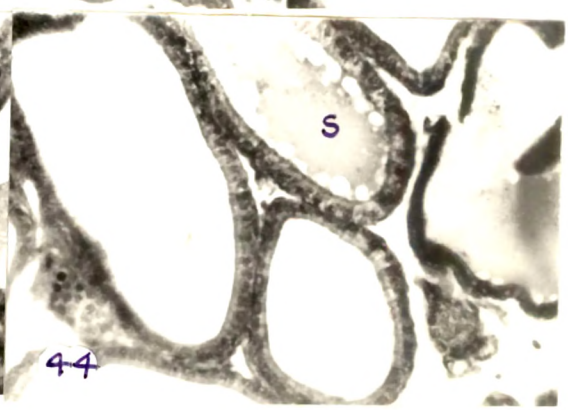
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PLATE VI

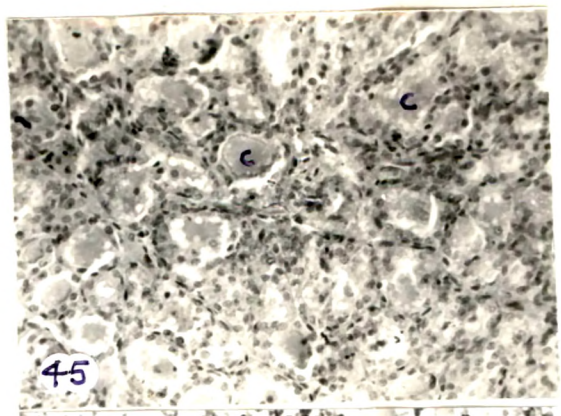
Figures 45-49: Photomicrographs of sections of thyroid gland of 35, 45, 60 and 90 day old HPOT +Px rats (200 x).

Figure 45: Section of thyroid of 35 day old rat showing follicles lined by cuboidal epithelium. Some follicles contain colloid (C).

Figure 46: Section of thyroid of 45 day old rat showing follicles with hypertrophied and vacuolated epithelium and narrow lumen with little colloid (C).

Figure 47: Section of thyroid of 60 day old rat showing follicles with hypertrophied epithelium, narrow lumen and little colloid (C).

Figures 48 and 49: Sections of thyroid of 90 day old rat showing follicles with hypertrophied epithelium and narrow lumen with moderate colloid (C) content.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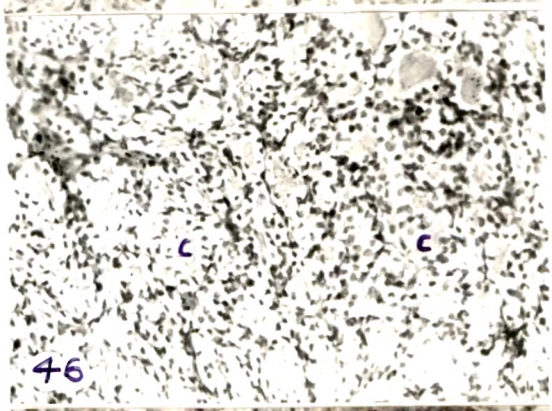
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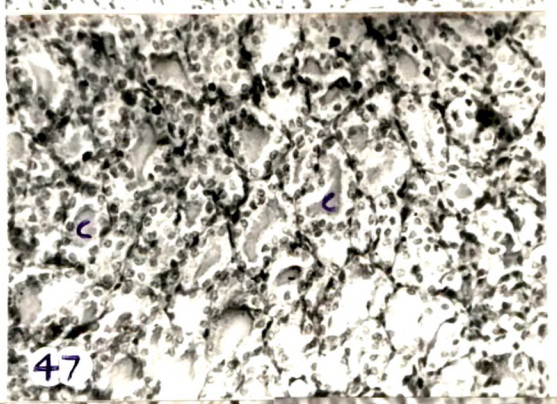
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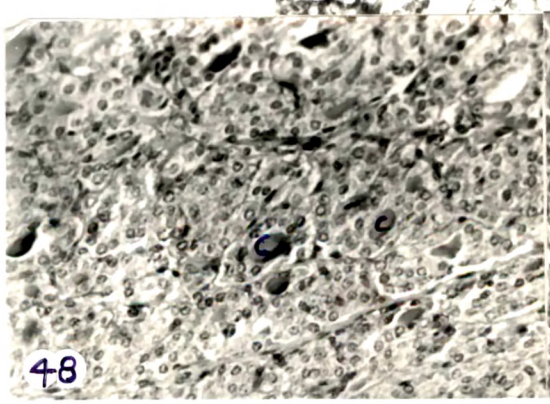
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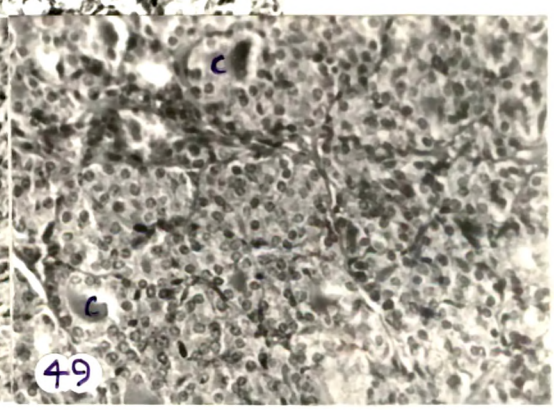
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HPOT + Px: The follicles were lined by low cuboidal epithelium with some of the follicles containing colloid.

45 Day Old

Control: The thyroid appeared less active with the follicles filled with colloid.

HPOT + Px: The follicular epithelium was hypertrophied and vacuolated with narrow lumen and little colloid.

60 Day Old

Control: The follicular epithelium appeared hypertrophied and the follicles contained low to moderate amount of colloid.

HPOT + Px: The follicular epithelium was hypertrophied and the follicles had narrow lumen and less colloid.

90 Day Old

Control: The follicles were lined by cuboidal epithelium and were full of colloid.

HPOT + Px: The follicular cells were hypertrophied and the follicles showed narrow lumen with moderate colloid content.

III. HISTOCHEMICAL OBSERVATIONS (Plates VII & VIII)

35 Day Old

In the testis of control animals, the 3ß-HSDH activity was clearly discernible in the Leydig cells and, there was a weak localisation in the tubules. The localisation in the Leydig cells was discernible with DHEA as the substrate but not with P. The 17ß-HSDH activity was appreciable in the tubules while no activity was visible in the Leydig cells of control rats. The 3α-HSDH activity was weakly localised in the tubules but not in the Leydig cells of control rats. Neither 3α, 3ß nor 17ß-HSDH activity could be noted in the interestial cells. There was noticeable activity

PLATE VII

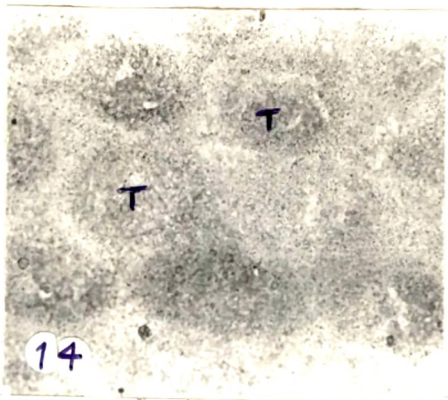
Figures 14-21: Photomicrographs of sections of testis of 35,45,60 and 90 day old HPOT + Px rats showing 3α and 17ß HSDH activities (65 x).

Figures 14 and 18: Sections of testis of 35 day old rat showing noticeable activity of both 3α HSDH (Fig.14) and 17ß HSDH (Fig.18) in the tubules (T) and no activity in the interstitium.

Figures 15 and 19: Sections of testis of 45 day old rat showing 3α HSDH (Fig.15) and 17ß HSDH (Fig.19) activity. Note the mild activity in the tubules (T) and almost no activity in the interstitium for 3α HSDH and noticeable activity in the interstitium (I) with moderate activity in the periphery of the tubules (T) for 17ß HSDH.

Figures 16 and 20: Sections of testis of 60 day old rat showing noticeable activity in the interstitium (I) and a very feeble activity in the tubule of 3α HSDH (Fig.16) and intense activity in the tubules (T) containing the advanced stages of germ cells and moderate activity in the interstitium (I) of 17ß HSDH (Fig.20).

Figures 17 and 21: Sections of testis of 90 day old rat showing mild activity of 3α HSDH (Fig.17) and 17ß HSDH (Fig.21) in the interstitium (I) and noticeable activity of 3α HSDH and strong activity of 17ß HSDH in the tubules (T), more prominent in the central part of the tubules containing advanced stages of germ cells.



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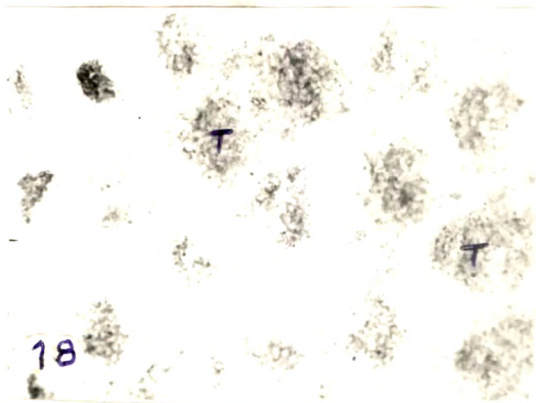

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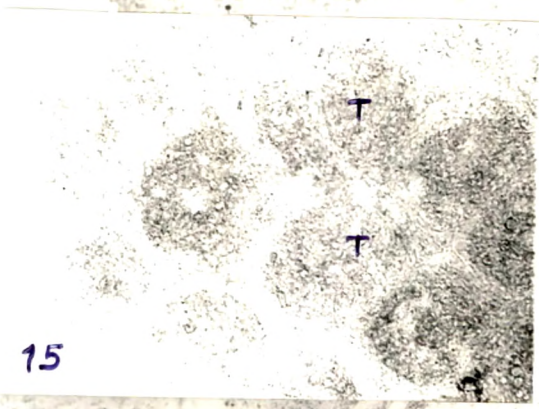

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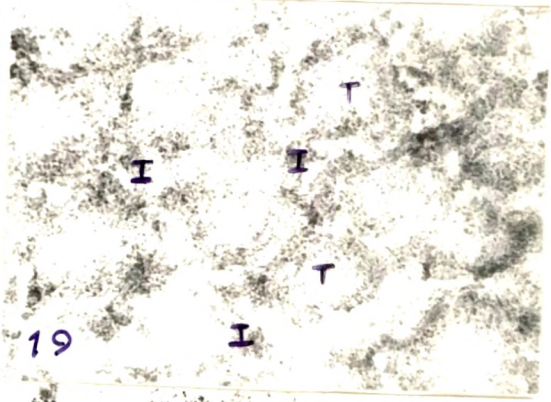

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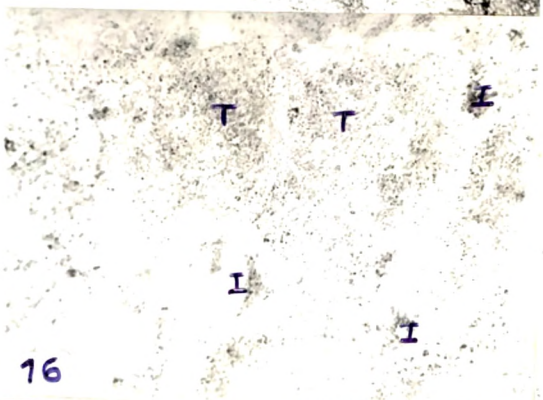

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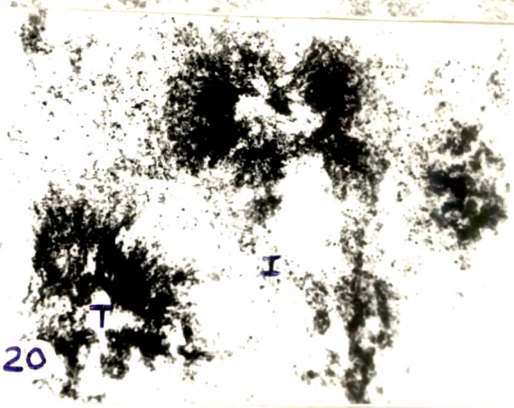

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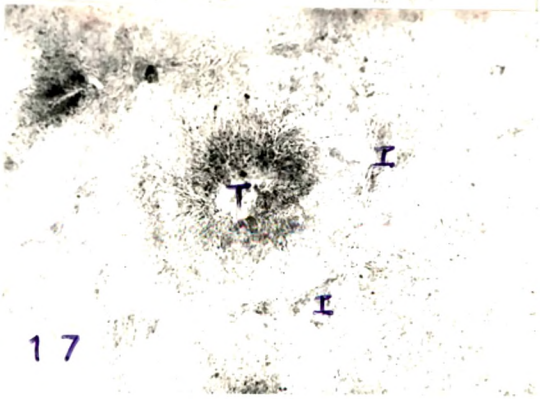

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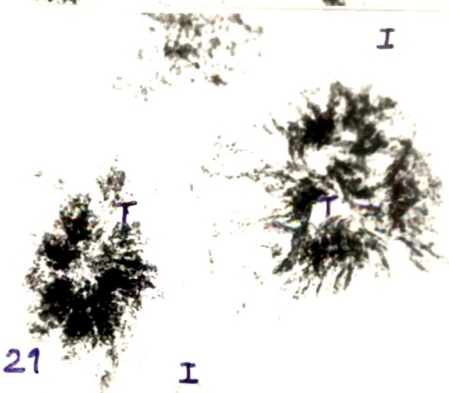

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PLATE VIII

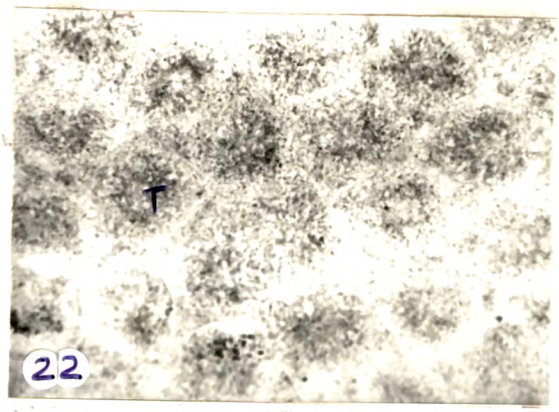
Figures 22-27: Photomicrographs of sections of testis of 35,45,60 and 90 day old HPOT + Px rats showing 3ß HSDH with P and DHEA as substrates (65 x).

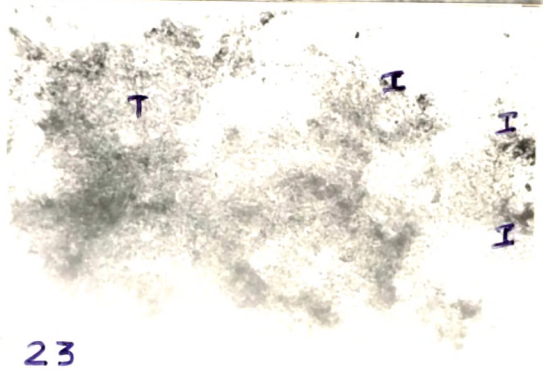
Figure 22: Section of testis of 35 day old rat showing mild activity in the tubules (T) with P as the substrate.

Figure 23: Section of testis of 45 day old rat showing noticeable activity in tubules (T) and strong localisation in the interstitium (I) with DHEA as the substrate.

Figures 24 and 26: Sections of testis of 60 day old rat showing week but noticeable activity in the interstitium (I) and almost no activity in the tubules with both the substrates.

Figures 25 and 27: Sections of testis of 90 day old rat showing prominent activity in the tubules (T) more with DHEA as the substrate (Fig. 27) and mild activity in the interstitium (I).





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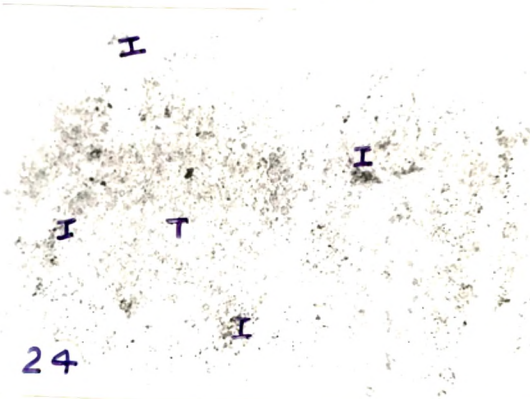

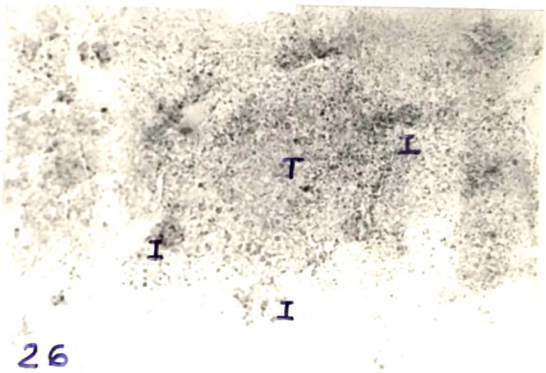

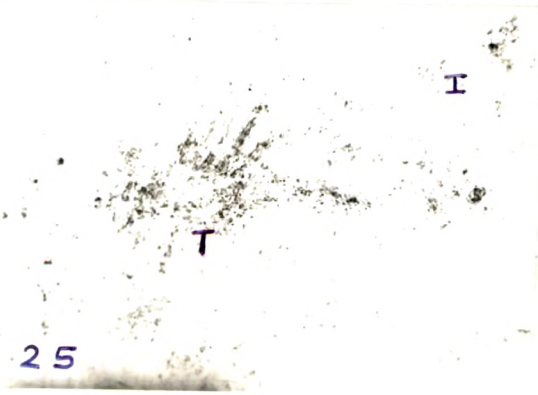

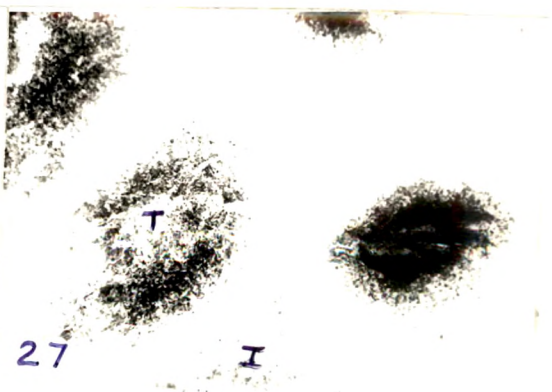

of both 3α and 17ß-HSDH in the tubules. 3ß-HSDH activity in the tubules showed a mild localisation with P as the substrate and no activity with DHEA.

45 Day Old

In the control rats, mild activity could be seen in the Leydig cells, noticeably more with DHEA as the substrate. The enzyme activity was reduced as compared to 35 day's. There was mild 3ß-HSDH activity in the Leydig cells with DHEA as substrate which was not evident with P. In the control animals, the Leydig cells were weakly enzyme active while the tubules showed significant activity. Though the 17ß-HSDH activity was localised uniformly within the tubules containing early stages of germ cells, the enzyme activity was localised more in the luminal part in the tubules containing advanced stages of spermatogenesis. The 3α-HSDH activity was mild though discernible in tubules as well as in the interstitium of the control animals. In the Px rats, the 3α-HSDH showed feeble activity in the interstitium and mild activity in the tubules. 3ß-HSDH activity showed strong localisation in the interstitium with DHEA as the substrate and a less strong response with P as the substrate. The interestial tissue appeared to be more when compared to controls. The enzyme activity was also seen in the tubules, though weakly with DHEA as the substrate. 17ß-HSDH activity showed a differential localisation in interstitium with cells in some areas showing weak activity and in others no activity. The tubules showed moderate reactivity towards the enzyme and a tendency for localization towards the periphery of the tubules was discernible.

60 Day Old

In the control rats, the 3ß-HSDH activity was very strong in the tubules and weak in interstitium with DHEA as the substrate. While the enzyme activity was more uniform in the tubules containing earlier stages, it was more intense in the luminal part in tubules containing advanced stages of germ cells. Relatively the enzyme activity was weak with P. Compared to 45 days, the enzyme activity was significantly more. In the control rats, the tubules were more 17ß-HSDH responsive than the interstitium, though the latter was also enzyme responsive. Compared with

3ß-HSDH, the enzyme activity was less at the same age. In the control, the 3α-HSDH activity was very strongly localised in the tubules, almost as intense as 3ß-HSDH activity. Whereas there was no activity of 3α-HSDH in the tubules, there was noticeable activity in the interstitium. There was noticeable 3ß-HSDH activity in the interstitium, more prominently with DHEA as the substrate. The activity appeared differential with some cells showing weak activity and some moderate. The tubules showed almost no activity except for a mild response by a few tubules with P. 17ß-HSDH activity was moderate to strong in the interstitium. The interestial tissue appeared to be more than the controls. There was almost no activity in the tubules except for weak activity in the central part of some tubules.

90 Day Old

In the controls, the 3ß-HSDH activity was weak in the tubules and appreciable in the interstitium. Comparatively, the enzyme activity was more intense with P as the substrate. In general, tubules with advanced stages of germ cells were enzyme responsive and the activity was more localised towards the luminal part. Compared to 60 days, the enzyme activity with DHEA as the substrate was much less while, with P, it was increased. The 17ß-HSDH activity was very strong and clearly discernible towards the luminal part containing advanced stages of germ cells in the testis of control animals. The interstitial cells were mildly enzyme responsive. Relatively, the 17ß-HSDH activity was more than that of 3ß-HSDH. The 3α-HSDH activity was very much reduced as compared to 60 days and the intensity and distribution was similar to that of 3ß-HSDH. There was noticeable activity of 3α-HSDH in the interstitium and mild activity in the central part of the tubules. 3ß-HSDH was moderate with DHEA as the substrate and noticeable with P as the substrate in the interstitium and in general, the intensity of the enzyme activity was more than the controls and the interstitial tissue appeared to be more than the controls. Tubules showed weak activity though, noticeable activity was discernible in the central part of those tubules containing the advanced stages of germ cells. The 17ß-HSDH activity was moderate both in the

interstitium and tubules and intense activity could be seen in the central part of the tubules containing advanced stages of germ cells.

IV. SERUM HORMONE PROFILE (Table 3.6; Fig. 12 a, b & c)

T_3 and T_4

Both T_3 and T_4 increased continuously in control rats from 35 days to reach peak levels at 60 days and then decreased by 90 days to the 35 day level. The HPOT + Px rats showed significantly lowered T_3 and T_4 levels at 35 days. Thereafter their titres increased to reach the highest level by 60 days, though not attaining the levels seen in control animals. Like the controls, the HPOT + Px rats also showed a decrease in the hormone levels between 60 and 90 days.

Testosterone

The serum T level increased in the control rats to peak level at 45 days. At 60 days the T level was reduced but then again increased to a higher level by 90 days, slightly less than the 45 day level. The serum T level remained low at 35 and 45 days in the HPOT + Px and then increased to a peak level at 60 days which was again followed by a decrease at 90 days. The changes seen between 45 and 90 days in the HPOT + Px animals appear to be similar to the changes seen between 35 to 60 days in the control rats.

DISCUSSION

Previous studies on Charles foster strain of rats showed that neonatal hypothyroidism has a retardatory influence on body and reproductive organ weights (chapter 1). However, neonatal Px exerted an overall positive influence and the body and reproductive organ weights and were significantly greater when compared to age matched controls (chapter 2). The present study executed to understand the combined effects of neonatal hypothyroidism and Px, has yielded results, some of which identify with that obtained individually for HPOT or Px while, others are quite distinct from either. The body weight of HPOT+Px animals remained significantly lesser

Table 3.6 Chronological alterations in Serum Triiodothyronine, Thyroxine and Testosterone levels in intact and hypothyroid rats subjected to pinealectomy (HPOT + Px)

Treatment	TRIIODOTHYRONINE (ng/mL)				THYROXINE (ng/mL)				TESTOSTERONE (ng/mL)			
	Age in Days				Age in Days				Age in Days			
	35	45	60	90	35	45	60	90	35	45	60	90
Control	2.57 ± 0.06@	2.90 ± 0.03	4.23 ± 0.10	2.43 ± 0.04	56.65 ± 3.09	75.76 ± 1.61	92.69 ± 7.34	54.89 ± 2.70	0.54 ± 0.18	1.77 ± 0.36	0.70 ± 0.21	1.44 ± 0.38
HPOT + Px	1.98 ± 0.29^{c}	1.99 ± 0.19^{d}	2.35 ± 0.33^{d}	2.10 ± 0.18^{ns}	42.03 ± 2.16^{d}	64.02 ± 4.71^{d}	72.00 ± 4.66^{d}	62.50 ± 4.25^{c}	0.48 ± 0.15^{ns}	0.51 ± 0.17^{d}	1.27 ± 0.22^{c}	0.75 ± 0.16^{d}

@ Values expressed as Mean ± SD of five experiments

^{c} $p < 0.01$; ^{d} $p < 0.001$; ^{ns} Not Significant

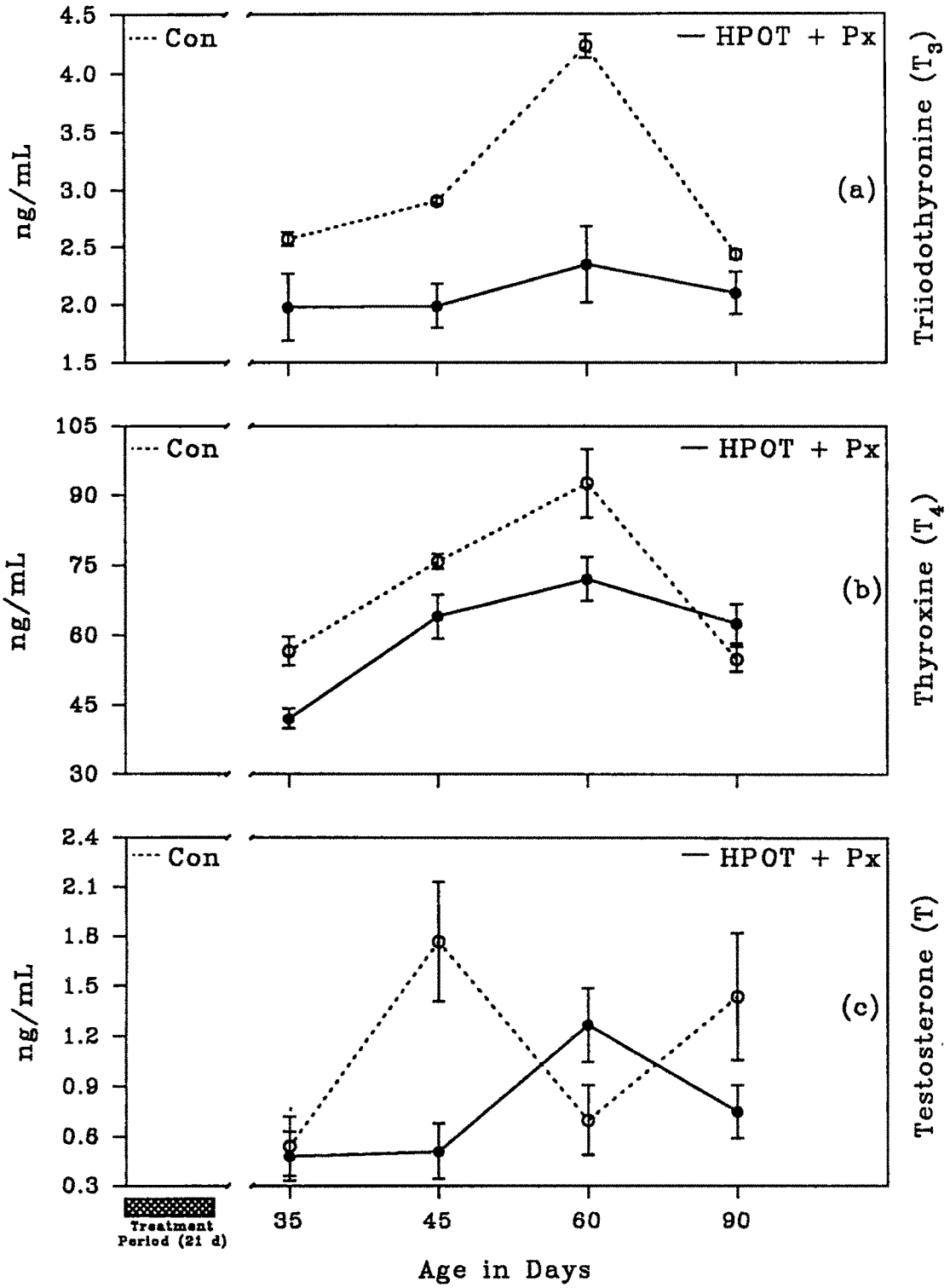

Fig. 12 (a, b & c) Chronological alterations in serum T_3, T_4 and T levels in hypothyroid rats subjected to pinealectomy (HPOT + Px)

than the controls at all periods. Interestingly, HPOT rats which had very low body weights till 60 days showed a tremendous catch-up growth thereafter to completely nullify the difference by 90 days. But in the present case, the HPOT+ Px animals, though weighed better than the HPOT animals till 60 days, did not show the type of rebound growth exhibited by the HPOT animals and hence they weighed significantly less than the controls even at 90 days. Apparently, the degree of growth retardation manifested in the early part seems to persist throughout as the HPOT+ Px animals which weighed 73% of the controls at 35 days weighed only 80% of the controls even at 90 days. In comparison, at the same age, while the HPOT rats equalled the weight of the controls, the Px rats surpassed the controls. A close scrutiny of the changes in body weight and serum thyroid hormone levels reveals that the maximum increase in percentage body weight is coincidentally preceded by a spurt in serum thyroxine level. In both control and HPOT+ Px animals the spurt occurred between 35 and 45 days and maximum growth between 45 and 60 days while in both the HPOT and Px animals the same occurred between 45 and 60 and 60 and 90 days respectively.

The paired weight of testes in HPOT + Px animals was 40% less than that of the controls at 35 days, but was nevertheless 254% more than that of HPOT animals (chapter 1). Between 35 and 45 days, there was retardation in testes growth which, ultimately led to a significant difference at 60 days compared to the controls despite, steady growth between 45 and 60 days. The testes of HPOT + Px animals weighed only 26% of the controls at 60 days but by 90 days they weighed 98% of the controls suggesting tremendous compensatory growth between 60 and 90 days. In the earlier study, it was seen that the HPOT rats also show a compensatory growth of testes between 60 and 90 days due to which the weight, which was only 10% of the controls at 60 days, recovered to 70% of the controls at 90 days (chapter 1). This indicates that Px can nullify the retardatory influence of neonatal hypothyroidism on the growth of testes by inducing a delayed compensatory growth at later periods. The favourable influence of neonatal Px on

testis growth was clearly shown in the earlier study which, could be seen in the early period itself and got amplified later (chapter 2).

The above morphometric changes find substantiation in the observed histological architecture and histochemical and hormonal profile of these animals. The testis of HPOT + Px animals showed reduced diameter of seminiferous tubules and had relatively more number of germ cells (mostly spermatogonia and very few spermatocytes) with fewer degenerating cells at 35 days compared to the controls. But by 45 days there was increase in germ cell degeneration and disruption of the cellular arrangement. Spermatogenic process was reestablished by 60 days with the orderly arrangement of germ cells and the appearance of spermatocytes and round spermatids. Spermatogenesis was fully established by 90 days with most of the tubules showing spermatids and spermatozoa. Comparatively, the tubules of HPOT + Px animals were more densely populated by all types of germ cells compared to the age matched controls. The tubular diameter though showed continuous increase, was nevertheless significantly lesser than that of controls at all times. Whereas in terms of germ cell population, the HPOT + Px animals resembles the Px animals, in terms of tubular diameter, they mimicked the HPOT animals. Inferably, thyroid hormone has some influence on tubular diameter as the final diameter attained in the adulthood is dependant on the initial spurt provided by the hormones. This is confirmed by the fact that transient neonatal hypothyroidism reduced the tubular diameter and this effect was sustained even after recovery from hypothyroidism as seen in the present, as well as the previous study (chapter 1). This is in contrast to the observations of Hess *et al.*(1993) and Meisami *et al.*(1994), of increased tubular diameter by neonatal hypothyroidism. This could be clearly accredited to a strain difference as had been inferred earlier from the many other features of the HPOT animals (chapter 1). However, the reduced diameter of the tubules might be compensated by increased length of the tubules as inferred earlier in Px animals (chapter 2) and as shown by FSH treatment (Meachem *et al.*, 1996).

The hormonal profile of HPOT + Px animals shows a clear hypothyroid state till 35 days and recovery to normal levels occurred only by 45 days. This is well compared with the histologically observed depleted colloidal content of the thyroid follicles at 45 days. This is quite similar to the Px animals (though the thyroid hormone levels were slightly higher than the Px animals) and in contrast to the HPOT rats whose serum thyroid hormone levels normalised only by 60 days. The profile of gonadotropic hormones could be marked by elevated FSH level and decreased LH and PRL, in a presumptive sense in HPOT + Px animals, which represents a mixed influence of hypothyroidism and Px. The increased germ cell proliferation specially of spermatogonia seen at 35 days can be related with increased FSH level. The increased germ cell population which could be seen at 90 days could also suggest a hyperproliferative effect of FSH on the Sertoli cells in the early periods (Cunningham *et al.*, 1978; Simorangker *et al.*, 1995; Meachem *et al.*, 1996; chapter 2). The tardy progression of spermatogenesis and increased germ cell degeneration seen at 45 days indicate inadequate support and /or conducive environment for the advanced stages of germ cells from pachytene stage onwards. In this respect the importance of both FSH and testosterone in supporting pachytene and post-pachytene stages of germ cells as well as in maintaining the process of spermatogenesis quantitatively and qualitatively has been established (Kerr *et al.*, 1992; McLachlan *et al.*, 1996). However, the lack of FSH or testosterone does not seem to be the cause for the present observation in HPOT + Px animals, as the level of FSH was supposedly elevated and that of testosterone not detrimental.

It is by now documented that increased thyroid hormone levels in the neonatal period induced triiodothyronine receptors in Sertoli cells to a maximum between 20 and 30 days whereby triiodothyronine and FSH together exert a synergistic effect in inducing Sertoli cell differentiation and maturation (Palmero *et al.*, 1989; 1995; Van Haaster *et al.*, 1992; 1993; Cooke *et al.*, 1994). From the present results on the thyroid hormone and FSH levels, it is apparent that there is subnormal thyroid hormone level till 45 days hence, a logical conclusion that can be

drawn is that, in the absence of adequate or optimal levels of thyroid hormones, Sertoli cell differentiation and maturation are delayed despite, the presence of FSH. Obviously, spermatogenesis cannot proceed to the post-meiotic stages without the structural and functional support rendered by the differentiated Sertoli cells. This aspect of the requirement for differentiated Sertoli cells and their functions in supporting advanced stages of germ cells is clearly documented by many previous reports (Kerr *et al.*, 1992; McLachlan *et al.*, 1996).

A similar delay in spermatogenesis seen in neonatally pinealectomised animals (chapter 2) as well as still longer delay seen in HPOT animals (chapter 1) could also support this inference as the time periods at which spermatogenesis had progressed to advanced stages increminatingly occurred subsequent to normalisation of thyroid hormone levels in all these experimental animals. Apparently, increased number of Leydig cells observed histologically and histochemically may, be related with a possible inhibitory influence of melatonin on Leydig cell proliferation in immature stages as reviewed earlier. This is supported by the recent reports of presence of melatonin receptors on Leydig cells (Valenti *et al.*, 1995). Obviously, removal of the inhibitory influence of melatonin as by Px in the present study could lead to increased proliferation of Leydig cells. However, the decreased testosterone production despite the increased number of Leydig cells seems to be a consequent effect of hypothyroidism. In this context, decreased basal and LH stimulated release of testosterone has been demonstrated in immature rats (Antony *et al.*, 1995) and also as inferred by us earlier (chapter 1).

Like that of testes the accessory glands also showed a retarded growth up to 60 days in the HPOT+ Px rats. At 35 days, while the weight of the seminal vesicle was significantly less, that of prostate and epididymis was comparable to that of controls. In the previous studies on neonatal hypothyroidism or Px, the weights of the accessory glands were found to be significantly less in the former group and identical to the controls in the later groups. The observed weights of the accessory organs in the present study are very much similar to those

noted in the previous study on Px animals. A common feature in all the three experimental groups is, the low titer of circulating thyroid hormone levels in the preweanling period. Based on this, the significantly lower weights of the accessory glands in the HPOT animals was accredited to a lack of growth promoting influence of thyroid hormones. But looking to the near or near normal weights of all three accessory glands in the Px and HPOT + Px animals, despite the low titers of thyroid hormone levels, the above explanation stands unjustified. Interestingly, some previous studies on chemical Px or melatonin administration in the preweanling period had shown sensitivity towards melatonin in terms of organ weights and metabolic physiology (Patel and Ramachandran, 1992; 1993). The observations thereat tended to suggest a generalised growth retardatory influence of melatonin and growth stimulatory influence in the absence of melatonin. The present observations seem to buttress the above and lend credence to the concept of growth retarding influence of melatonin in the neonatal period. Obviously, hypothyroidism in a pineal intact neonate is more detrimental to the growth of the accessory reproductive organs. But hypothyroidism in pinealectomised neonates (in the absence of melatonin) seems to have no effect on the growth and weight of the accessory organs in the neonatal and juvenile periods. This would suggest that melatonin has definite growth retardatory influence in rats at least up to the prepubertal period and this effect is clearly manifested in the hypothyroid state. This is further emphasised by the present study wherein absence of melatonin despite the hypothyroid state maintained normal accessory organ weights. The growth promoting influence of Px is more marked in the epididymis as the histological observations revealed increased number of nuclei, cell height and tubular diameter at 35 days.

In the control animals, maximum growth and histologically visible differentiation and maturation of the accessory glands occurred between 45 and 60 days while, there was no such growth effect in the HPOT + Px animals at this period. This distinct difference is related to the hormonal profile. In the control animals, there is increased availability of PRL and testosterone during this period, both of which act synergistically to control the postpubertal growth,

differentiation and functional maturation of the accessory glands. In this connection the importance of androgen and PRL in regulating the growth and functions of accessory glands, especially the prostate is well documented (Shannon and Cunha, 1983; Kharroudi and Slaunwhite, 1984; Takeda *et al.*, 1985; Husmann *et al.*, 1991; chapters 1 and 2). Apparently, in the HPOT + Px animals, there is a sluggishness in the elevation of peripheral testosterone levels and delayed normalisation of PRL levels, which have resulted in a retarded growth of the glands between 45 and 60 days. However, with the availability of adequate PRL and testosterone after 60 days, the accessory glands show a delayed hypersensitive response marked by a tremendous growth spurt resulting in attainment of weights of epididymis and seminal vesicle comparable to the controls and, that of prostate, much greater than the controls by 90 days.

Overall, the results of the present study reveal that a delayed testicular maturation and establishment of spermatogenic functions occur mainly due to the inability of FSH to induce Sertoli cell differentiation in the absence of potent levels of thyroid hormones. Therefore, a synergistic action of both thyroid hormone and FSH as well as the essentiality of thyroid hormone in inducing Sertoli cell differentiation are envisaged. There is also increased germ cell production without an actual increase in the diameter of the tubules essentially due to the hyperproliferative action of FSH on Sertoli cells in the absence of differentiation inducing influence of thyroid hormones. There is also an apparent Leydig cell hyperplasia due to the increased sensitivity of the Leydig cells towards LH in the absence of melatonin. A growth retarding influence of melatonin on sex accessory glands is also revealed.