CHAPTER 5

TRANSIENT NEONATAL HYPERTHYROIDISM AND PINEALECTOMY INCREASES GERM CELL NUMBER AND INDUCES DIFFERENTIAL ACCESSORY ORGAN GROWTH IN THE RAT: POSSIBLE PINEAL, THYROID AND GROWTH HORMONE INTERACTIONS.

Early studies of thyroid on the male reproductive system and on the functions of testis found the role of thyroid to be inconsequential (Hammet, 1923; Bruni *et al.*, 1975; Kalland *et al* .,1978; Del Rio *et al.*, 1979). Subsequent studies on hypothyroidism reported either inhibitory effects or no effects at all (Hammet, 1923; Del Rio *et al.*, 1979). Studies on gonadotropins and testosterone (T) in hypothyroid animals also produced controversial results with some showing changes in gonadotropins and T levels (Baksi, 1973; Bruni *et al.*, 1975), while others showing no change (Kalland *et al.*, 1978). Over the years, the experimental observations on animal models tended to indicate relative insensitivity of the adult condition towards thyroid hormone status; however, there were indications that testicular functions may be more affected in the immature stage. Recent studies conducted on these lines showed localization of T_3 receptors in testis (Palmero *et al.*, 1988, 1992) and both *in vivo* and *in vitro* studies demonstrated that thyroid hormone can directly regulate the early postnatal development of rat testis. *In vivo* administration of T_4 in neonatal animals or cultured testis fragments in presence of thyroid hormone, demonstrated stimulatory effects, such as gonocyte proliferation, increase in seminiferous cord diameter and testis size (Amin and El-Sheikh, 1977; Chowdhury *et al.*, 1984; Van Haaster *et al.*, 1993; Jannini

et al., 1993). However, more interesting observations were made when rats were rendered hypothyroidic neonatally. Controlled experimental studies on these lines showed that induction of transient hypothyroidism by goitrogenic compounds in the neonatal condition can result in enlarged adult testes size and that, this effect is restricted to a critical period of 21 days after birth (Cooke *et al.*, 1992; 1993). Though these observations were made in Long-Evans and Sprauge-Dawley strains of rat, similar studies conducted on Charles-foster strain resulted in a paradoxically reduced adult testicular size (chapter 1). An apparent strain difference was considered to be responsible for the differential response. In this context, a corollary experiment on transient neonatal hyperthyroidism in this strain also resulted in adult testis size similar to the hypothyroid animals (chapter 4). A similar effect of hyperthyroidism was also reported in Wistar strain of rats (Van Haaster *et al.*, 1993).

Unlike neonatal hypothyroidism, neonatal Px in Charles-foster strain resulted in hypertrophied adult testis and sex accessory organs (chapter 2). The above observations in pinealectomised animals was attributed to the hyperproliferative influence of elevated FSH on Sertoli cells in the absence of a permissive influence of thyroid hormones (as Px reduced T_4 and T_3 levels) on Sertoli cell differentiation. In contrast, neonatal hyperthyroidism reduced gonadotropin levels and also, thyroid hormones, subsequent to withdrawal of T_4 administration. These set of changes were purported to exert a delayed and shortened period of Sertoli cell proliferation, immediately followed by their differentiation, leading to reduced testis size (chapter 4). In the wake of the observed effects of hyperthyroidism and Px, it was tempting to test the combined effect of neonatal Px and hyperthyroidism. The present study has therefore tried to evaluate the effect of transient neonatal hyperthyroidism in pinealectomised rats.

RESULTS

I. MORPHOMETRIC OBSERVATIONS

BODY WEIGHT (Table 5.1; Fig. 1 & 2)

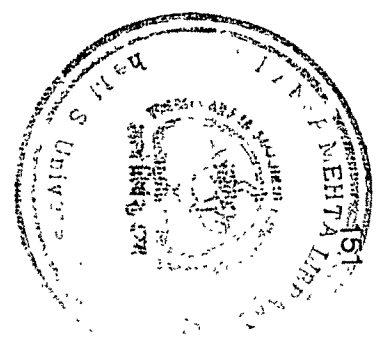
The HPRT + Px animals showed significantly lesser body weight compared to controls at 35

Table 5.1 Chronological alterations in Body Weight (gm), Percentage Difference and Per Day Growth Rate in intact and hyperthyroid rats subjected to pinealectomy (HPRT + 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
HPRT + Px	41.00 ± 5.74^{c}	48.30 ± 4.95^{a}	76.90 ± 3.80^{d}	106.08 ± 5.02^{b}	+ 17.80	+ 59.21	+ 37.95	+ 158.73	1.17 ± 0 09^{b}	0.73 ± 0 05^{d}	1.91 ± 0.12^{b}	0.97 ± 0.07^{ns}

@ Values expressed as Mean ± SD of five experiments

^{b} $p < 0.25$; ^{c} $p < 0.01$; ^{d} $p < 0.001$; ^{ns} Not Significant



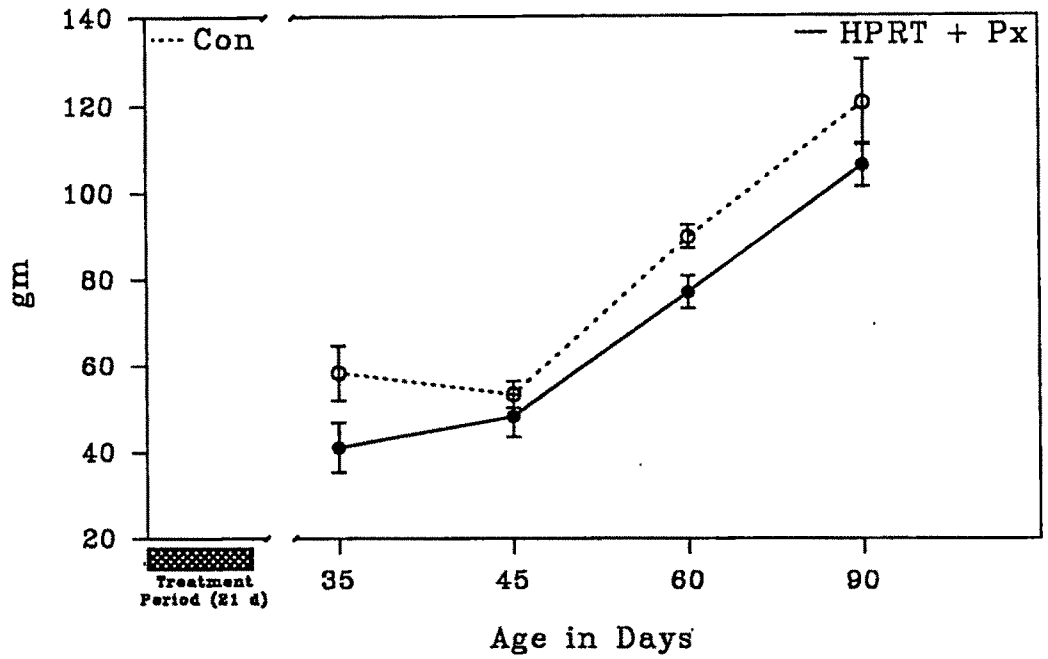


Fig. 1 Chronological alterations in body weight of hyperthyroid rats subjected to pinealectomy (HPRT + Px)

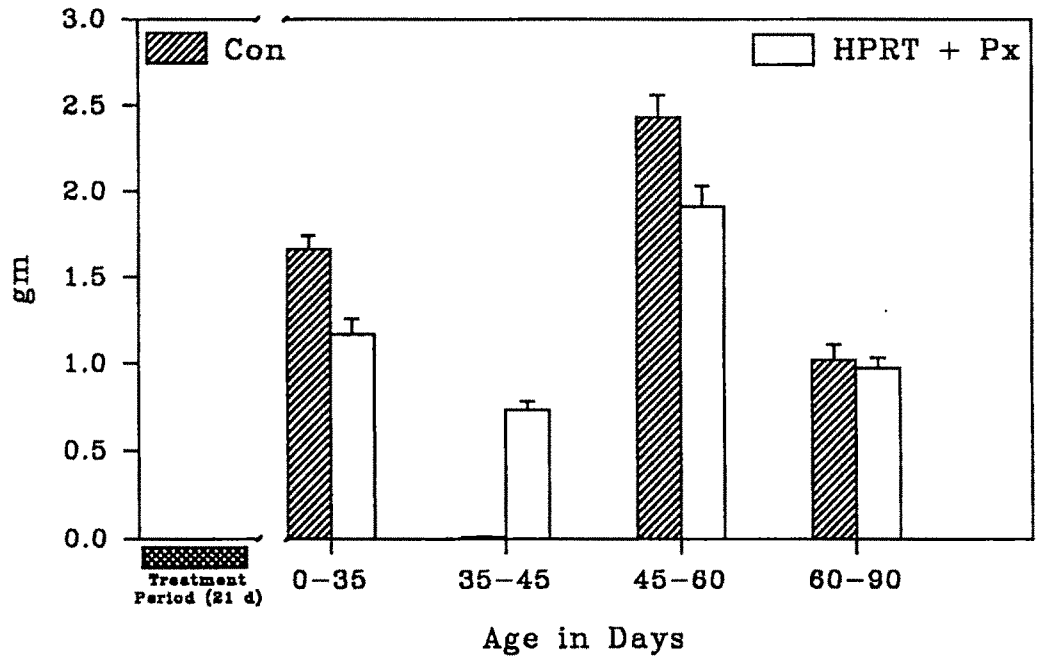


Fig. 2 Per day body growth in intact and HPRT + Px rats

days. Whereas the control animals showed a decrement in body weight at 45 days, the HPRT + Px animals showed continuous increment in body weight without decrease at any stage. The body weight at 90 days was less than the controls in HPRT + Px rats. Both the control and HPRT + Px animals showed maximum percentage increment in body weight between 45 and 60 days.

ORGAN WEIGHTS

Testes (Table 5.2 a, b; Fig. 3 a, b & 4)

At 35 days the testes weight was significantly less in HPRT + Px animals compared to the controls. The testes weight in control rats increased continuously from 35 to 90 days while, the HPRT + Px rats showed a decrease at 45 days. Thereafter, they showed continuous and steady increase in testes weight to attain similar testes weight at 90 days. On a percentage basis both the control and HPRT + Px rats showed maximum growth between 45 and 60 days. The relative weight showed continuous increase from 35 to 90 days in control animals, the HPRT + Px rats recorded decreased relative weight at 45 days. The maximum relative testes weight was shown by HPRT + Px rats at 90 days compared to the controls.

Epididymis (Table 5.2 a, b; Fig. 5 a, b & 6)

The absolute weight of epididymis was significantly lower in HPRT + Px group of rats as compared to the control animals at 35 days. The control animals showed a transient decrement in weight at 45 days, the HPRT + Px animals recorded continuous increment. At 90 days the weight of the epididymis was greatly reduced in HPRT + Px animals as compared to the controls. The maximum percentage growth of epididymis in both control and HPRT + Px animals occurred between 45 and 60 days. In general, the relative weight of epididymis showed progressive increase from 35 to 90 days in both control and HPRT + Px animals with a decrease at 45 days. The relative weight at 35 days was significantly increased in control animals compared to the HPRT + Px animals.

Table 5.2 (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 hyperthyroid rats subjected to pinealectomy (HPRT + 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
	HPRT + Px	428.20^{b} ± 6.18	391.70^{d} ± 10.77	1105.80^{d} ± 77.47	2472.72^{ns} ± 67.87	1.06^{b} ± 0.16	0.82^{d} ± 0.06	1.44^{a} ± 0.11	2.33^{d} ± 0.09
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
	HPRT + Px	54.96^{d} ± 4.58	65.38^{ns} ± 3.99	180.10^{d} ± 13.21	396.01^{d} ± 13.82	0.14^{c} ± 0.02	0.14^{b} ± 0.01	0.23^{d} ± 0.02	0.37^{c} ± 0.02

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
	HPRT + Px	- 8.52	+ 182.31	+ 123.61	+ 477.47	12.23^{ns} ± 1.05	-	47.61^{ns} ± 5.68	45.56^{c} ± 5.02
EPIDIDYMIS	Con	- 47.68	+ 513.56	+ 27.75	+ 310.09	3.51 ± 0.32	-	22.06 ± 6.45	3.65 ± 0.24
	HPRT + Px	+ 18.96	+ 175.47	+ 119.88	+ 620.54	1.57^{d} ± 0.09	1.04^{d} ± 0.09	7.65^{d} ± 0.19	7.19^{d} ± 1.02

@ Values expressed as Mean ± SD of five experiments; ^{a} $p < 0.05$; ^{b} $p < 0.025$; ^{c} $p < 0.01$; ^{d} $p < 0.001$; ^{ns} Not Significant

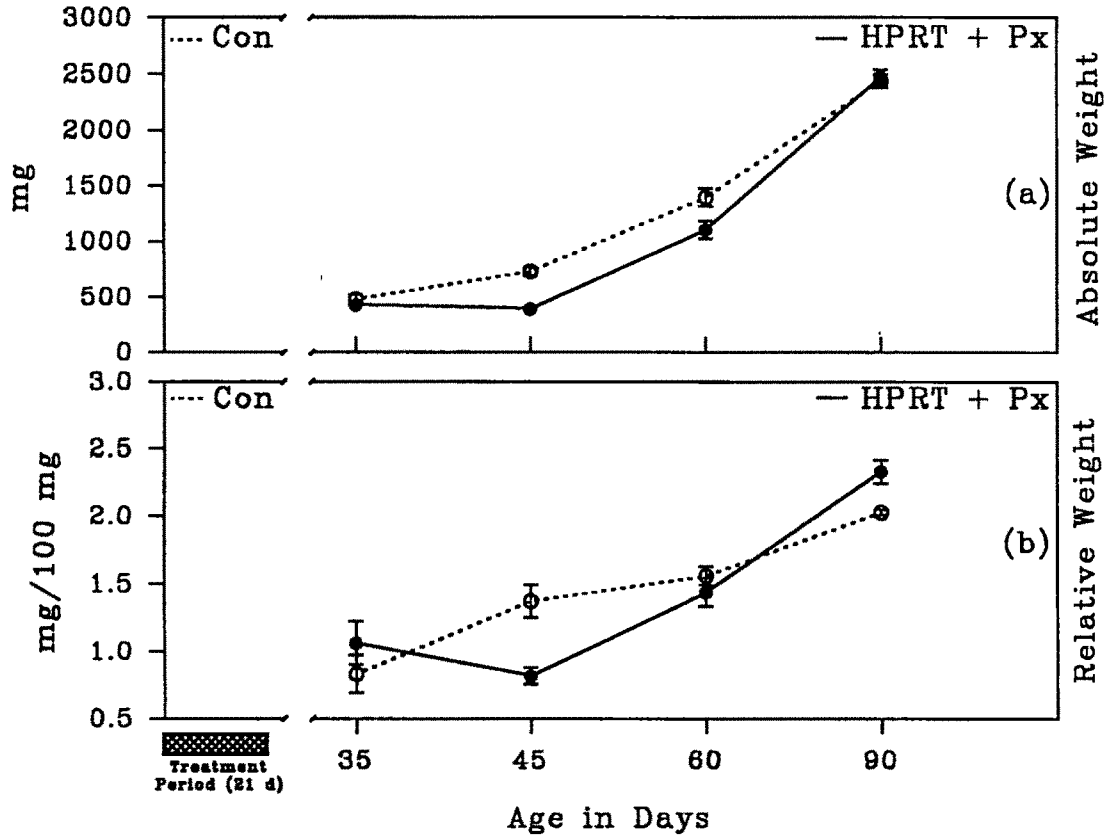


Fig. 3 (a&b) Chronological alterations in weights of testes in hyperthyroid rats subjected to pinealectomy (HPRT + Px)

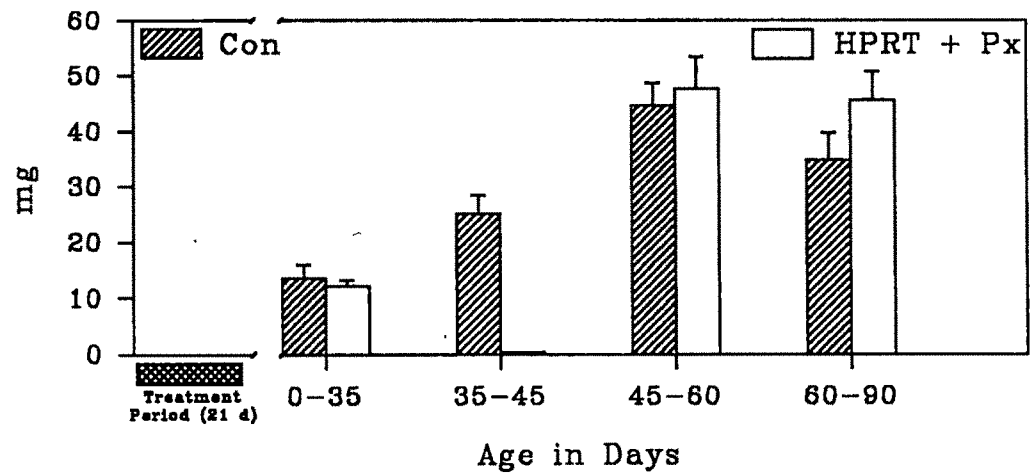


Fig. 4 Rate of growth of testes in intact and HPRT + Px rats

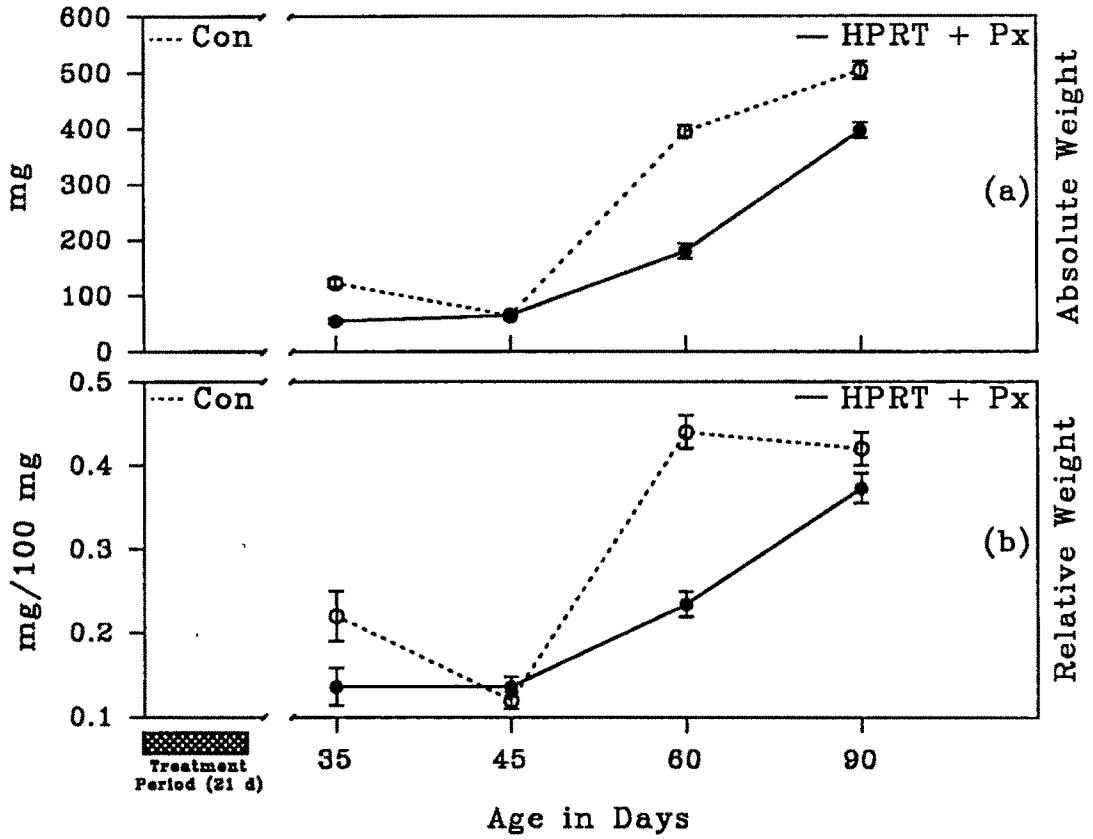


Fig. 5 (a&b) Chronological alterations in weights of epididymis in hyperthyroid rats subjected to pinealectomy (HPRT + Px)

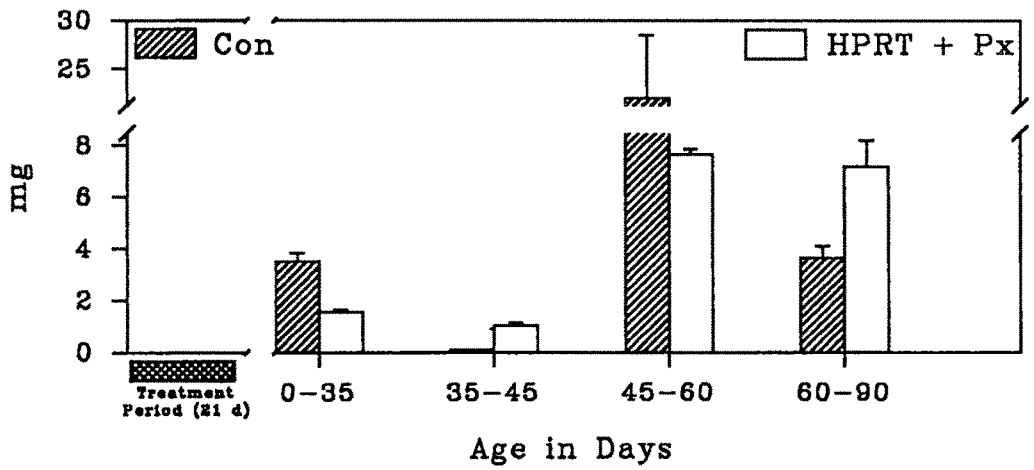


Fig. 6 Rate of growth of epididymis in intact and HPRT + Px rats

Seminal Vesicle (Table 5.3 a, b; Fig. 7 a, b & 8)
The weight of seminal vesicle was similar in both control and HPRT + Px rats at 35 days. Both the control and HPRT + Px animals showed a decrement in-between at 45 days (more pronounced in the latter), before recording continuous increment. At 90 days, the weight of seminal vesicle was slightly higher in HPRT + Px animals compared to that of the controls. The percentage increment in the weight of seminal vesicle was the greatest between 45 and 60 days in control animals while the HPRT + Px animals showed higher growth rate both between 45 and 60 and 60 and 90 days. The relative weight of seminal vesicle at 35 days was significantly high in HPRT + Px animals compared to the control rats. There was a decrement in relative weight in both the groups of rat at 45 days, more pronounced in HPRT + Px group and, at 90 days, the relative weight was significantly high in HPRT + Px group of animals.

Prostate Gland (Table 5.3 a, b; Fig. 9 a, b & 10)
The HPRT + Px rats showed significantly higher weight of prostate at 35 days compared to the controls. There was a decrease in the weight of the prostate at 45 days in both the control and HPRT + Px animals before, showing continuous increment. At 90 days, the final weight of prostate was similar in both the control and HPRT + Px groups of rat. The maximum percentage increase in prostate weight occurred between 45 and 60 days in control animals, while, the same occurred between 60 and 90 days in HPRT + Px animals. In general, the relative weight of prostate paralleled the changes in absolute weight.

Thyroid Gland (Table 5.4; Fig. 11 a, b)
The absolute weight of thyroid in HPRT + Px animals was significantly reduced at 35 days compared to the controls. Though the HPRT + Px animals showed continuous increase in thyroid weight thereafter, the control animals showed a decrement in-between at 45 days. The maximum percentage increase in thyroid weight occurred between 45 and 60 days in controls, while the same occurred in HPRT + Px animals between 60 and 90 days. The relative weight was high

Table 5.3 (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 hyperthyroid rats subjected to pinealectomy (HPRT + 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
	HPRT + Px	25.75^{ns} ± 4.65	16.30^{ns} ± 2.59	88.55^{d} ± 5.68	490.88^{d} ± 10.37	0.065^{c} ± 0.016	0.034^{ns} ± 0.004	0.115^{d} ± 0.006	0.462^{d} ± 0.009
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	0.09 ± 0.009	0.128 ± 0.003
	HPRT + Px	36.84^{c} ± 4.76	18.81^{c} ± 2.21	41.62^{d} ± 5.72	149 19^{ns} ± 12.63	0.092^{d} ± 0.023	0.039^{d} ± 0.004	0.053^{d} ± 0.006	0.141^{d} ± 0.005

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
	HPRT + Px	- 36.69	+ 443.25	+ 454.35	+ 1806.33	0.74^{ns} ± 0.02	-	4.82^{b} ± 0.46	13.41^{b} ± 1.98
PROSTATE GLAND	Con	- 37.94	+ 435.51	+ 89.87	+ 531.03	0.69 ± 0.09	-	4.41 ± 0.95	2.43 ± 0 89
	HPRT + Px	- 48.94	+ 121 26	+ 258.46	+ 304.96	1 05^{a} ± 0.10	-	1.52^{d} ± 0.10	3.58^{c} ± 0 15

@ Values expressed as Mean ± SD of five experiments; ^{a} $p < 0.05$; ^{b} $p < 0.025$; ^{c} $p < 0.01$; ^{d} $p < 0.001$; ^{ns} Not Significant

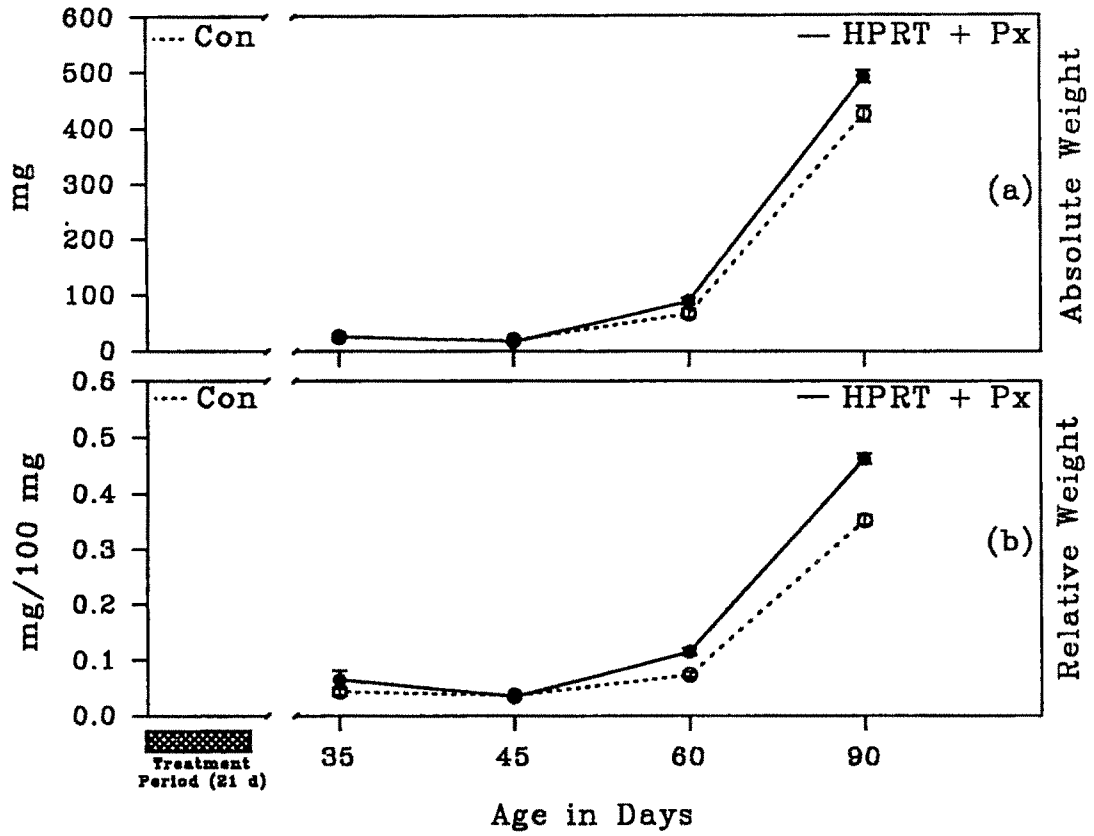


Fig. 7 (a&b) Chronological alterations in weights of seminal vesicle in hyperthyroid rats subjected to pinealectomy (HPRT + Px)

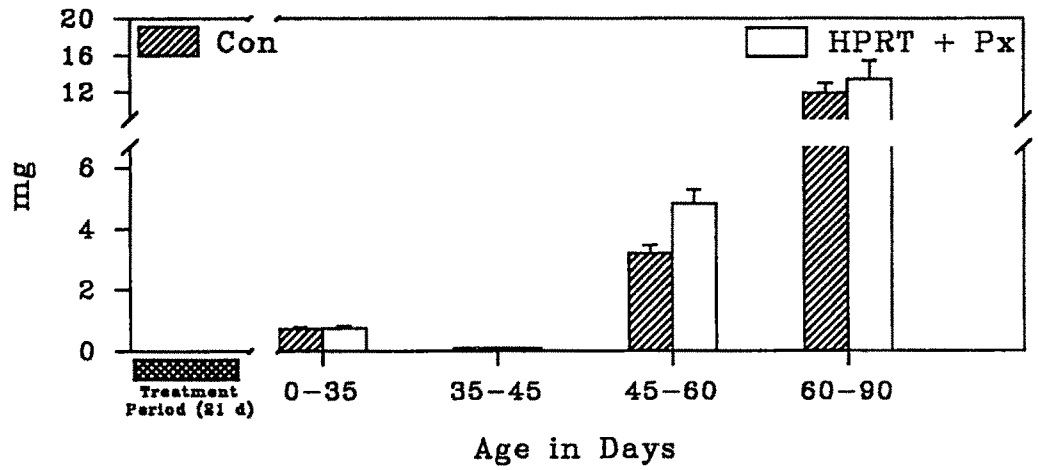


Fig. 8 Rate of growth of seminal vesicle in intact and HPRT + Px rats

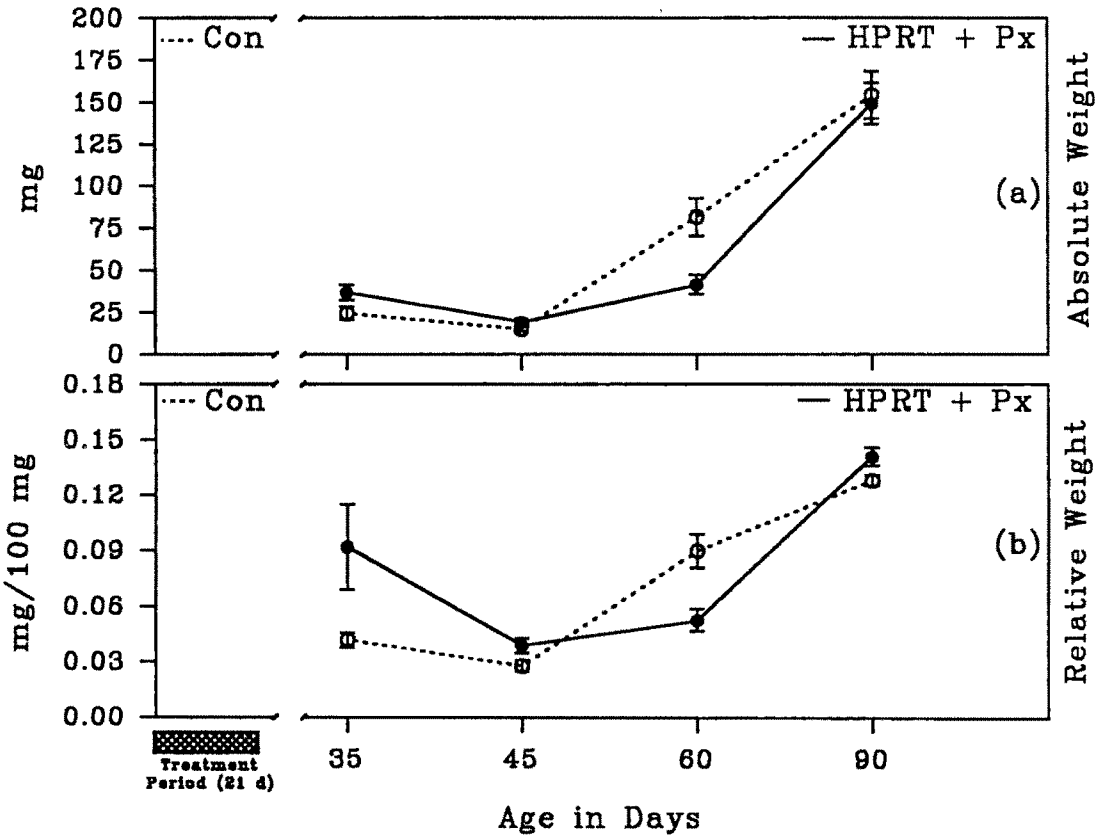


Fig. 9 (a&b) Chronological alterations in weights of prostate gland in hyperthyroid rats subjected to pinealectomy (HPRT + Px)

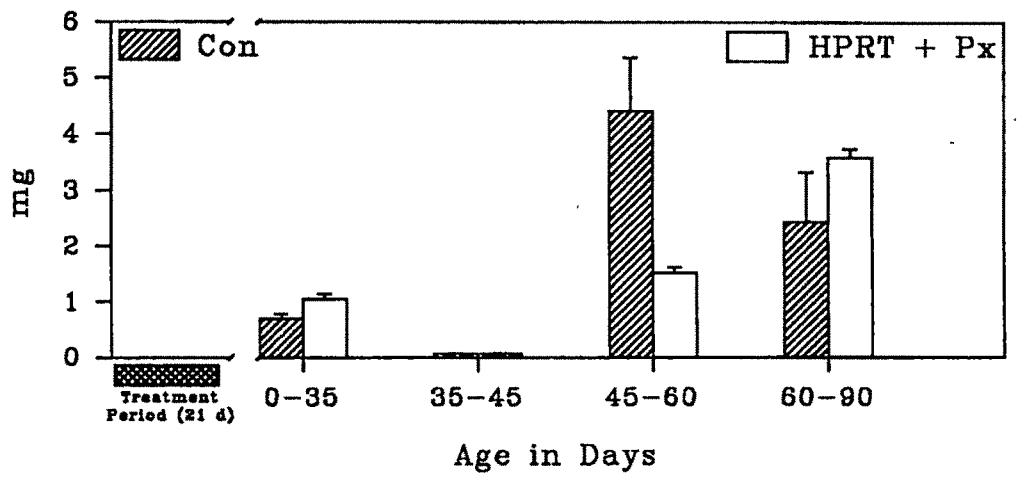


Fig. 10 Rate of growth of prostate gland in intact and HPRT + Px rats

Table 5.4 Chronological alterations in Weight [Absolute (mg) and Relative (mg/100mg)] and Percentage Difference of Thyroid Gland in intact and hyperthyroid rats subjected to pinealectomy (HPRT + 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
HPRT + Px	3.90 ± 0.52^{d}	4.22 ± 0.55^{ns}	5.42 ± 0.54^{c}	14.78 ± 2.12^{d}	0.009 ± 0.001^{ns}	0.009 ± 0.001^{c}	0.007 ± 0.001^{ns}	0.013 ± 0.002^{d}	+ 8.20	+ 28.44	+ 172.69	+ 278.97

@ Values expressed as Mean ± SD of five experiments

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

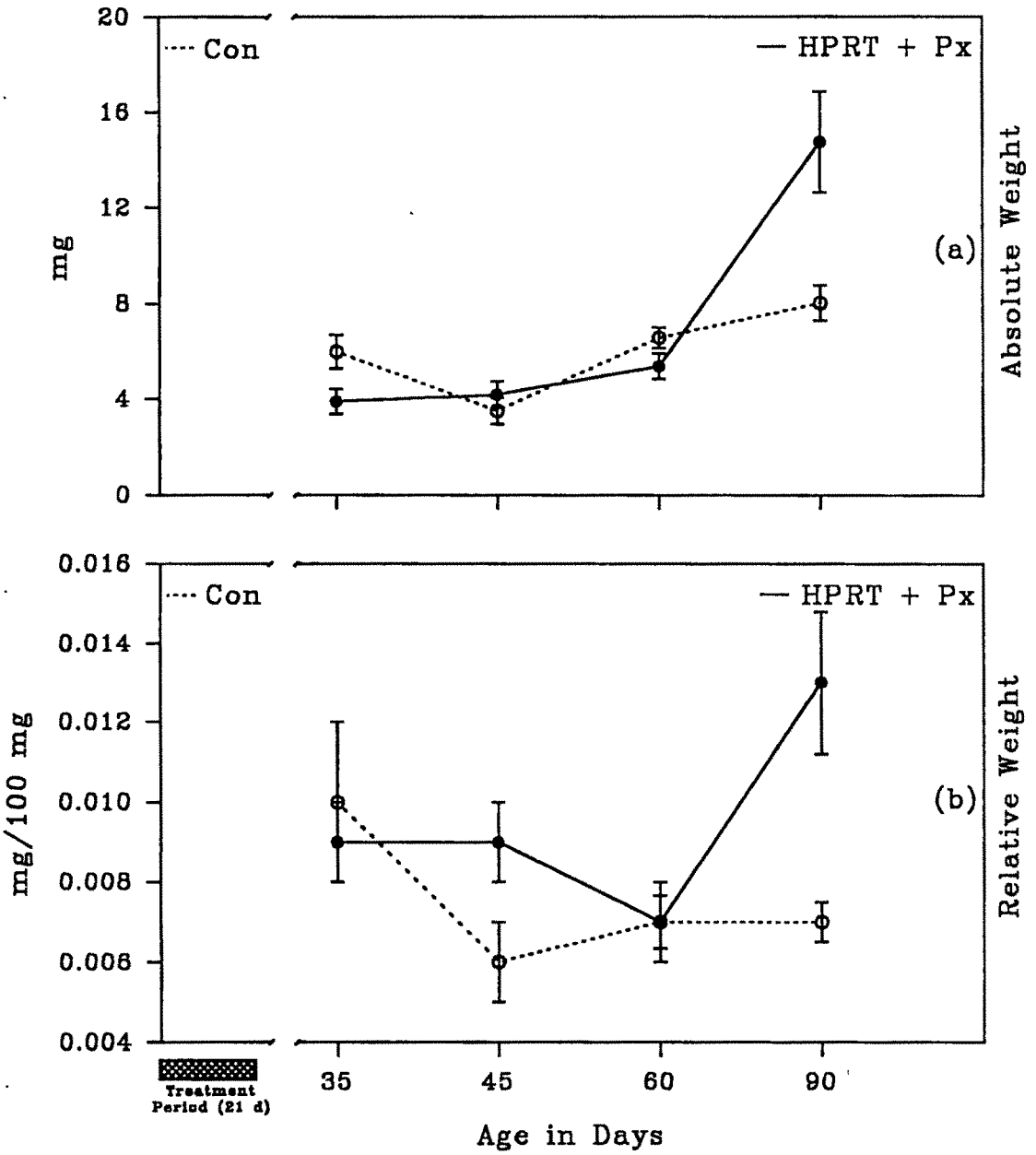


Fig.11 (a&b) Chronological alterations in weights of thyroid gland inperthyroid rats subjected to pinealectomy (HPRT + Px)

in control animals compared to the HPRT + Px animals at 35 days, the same was high in HPRT + Px animals compared to the controls at 90 days.

II. HISTOLOGICAL OBSERVATIONS

STRUCTURE OF TESTIS (Table 5.5; 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.

HPRT + Px: The tubules were smaller in size with an average diameter of 57.14 μm. There was reduced germ cell population and many of them appeared to be hypertrophied and showing signs of degeneration. Interstitium was poorly 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.

HPRT + Px: There was increase in tubular diameter and the degenerative changes were checked and spermatogenesis was reestablished with most of the tubules showing spermatocytes and round spermatids.

Table 5.5 Chronological alterations in the Diameter (in μm) of Seminiferous Tubule and Epididymis (Caput and Cauda) in intact and hyperthyroid rats subjected to pinealectomy (HPRT + 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
HPRT + Px	132.38 ± 7.17^{d}	140.56 ± 9.23^{c}	145.71 ± 10.43^{a}	188.09 ± 14.79^{ns}	25.57 ± 2.61^{ns}	30.81 ± 3.42^{b}	33.33 ± 3.72^{ns}	32.51 ± 3.62^{d}	18.90 ± 1.86^{d}	19.12 ± 2.03^{c}	20.86 ± 2.12^{ns}	16.33 ± 1.38^{b}

@ Values expressed as Mean ± SD of five experiments

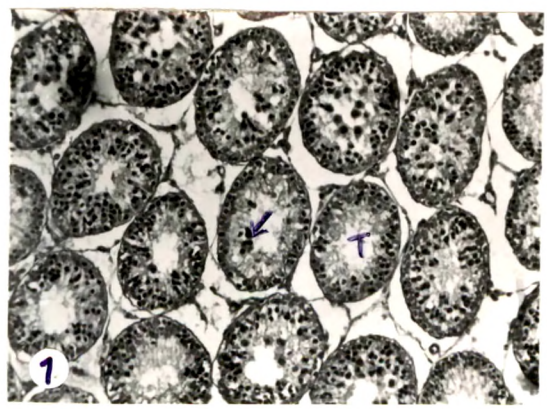
^{a} $p < 0.05$; ^{b} $p < 0.025$; ^{c} $p < 0.01$; ^{d} $p < 0.001$; ^{ns} Not Significant

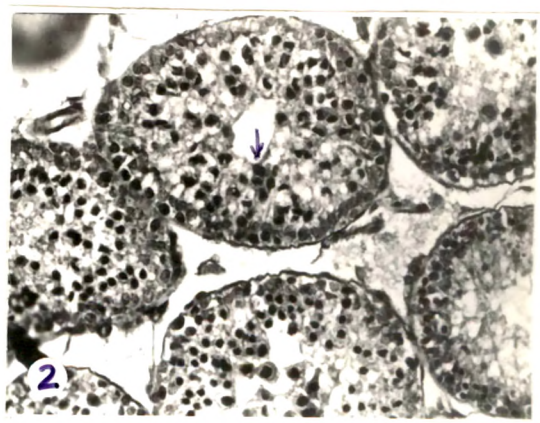
PLATE I

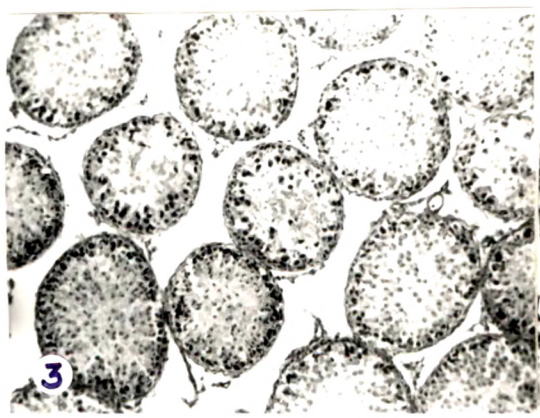
Figures 1-4: Photomicrographs of sections of testis of 35 and 60 day old neonatally hyperthyroid rats subjected to pinealectomy (HPRT + Px)

Figures 1 (100 x) and 2 (200 x): Sections of testis of 35 day old rat showing smaller tubules (T) with reduced germ cell population and many hypertrophied degenerating germ cells (arrow).

Figures 3 (100 x) and 4 (200 x): Sections of testis of 60 day old rat showing well formed tubules with compactly arranged germ cells. The overall germ cell population appears to be more.


1
T


2


3

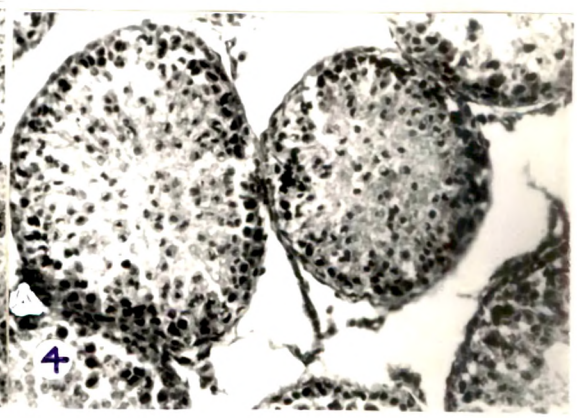
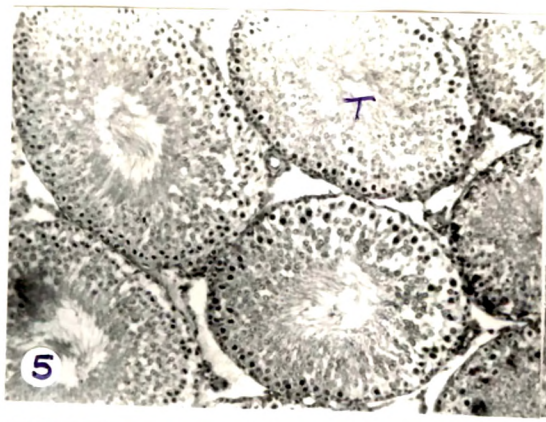

4

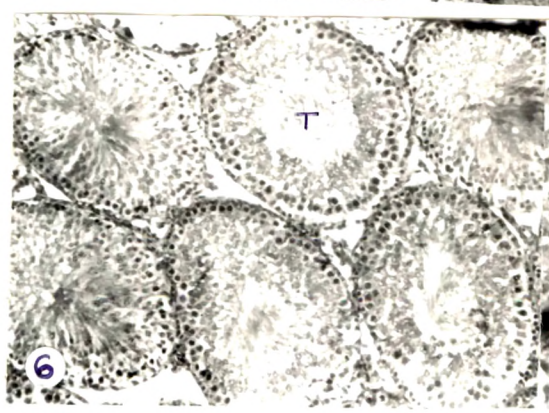
PLATE II

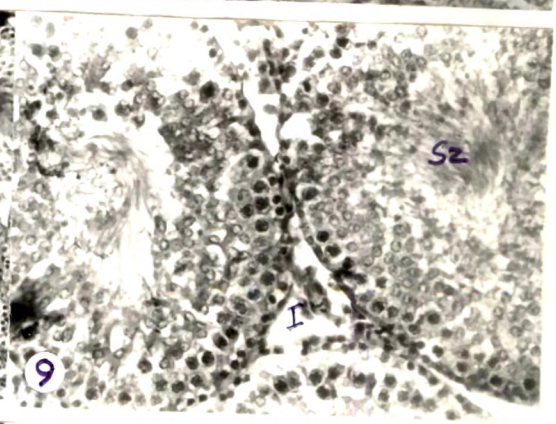
Figures 5-10: Photomicrographs of sections of testis of 90 day old neonatally hyperthyroid rats subjected to pinealectomy (HPRT + Px).

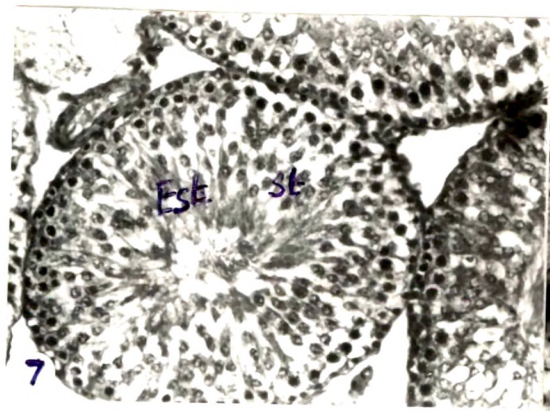
Figures 5 and 6 (100 x); 7-10 (200 x): Sections of testis of 90 day old rat showing well formed compactly arranged tubules with fully established spermatogenesis. Spermatids (St) and spermatozoa (Sz) could be seen in almost all the tubules. The interstitium (I) is prominent.

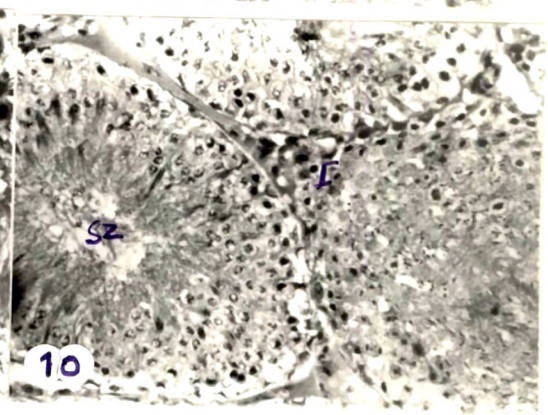

T
5


Sz
8


T
6


Sz
I
9


Est
St
7


I
Sz
10

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.

HPRT + Px: The tubules were well formed and well organised with an average diameter of 145.71 μm. The germ cells were also compactly arranged and elongated spermatids could be seen in many of the tubules. The overall germ cell population appeared to be increased. The interstitium appeared well formed.

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 appears to be moderately developed.

HPRT + Px: The tubules were well formed and compactly arranged and measured 188.09 μm in diameter. Spermatogenesis was fully established with all stages well represented. Almost all the tubules showed spermatozoa. The interstitium appeared prominent and well developed.

STRUCTURE OF EPIDIDYMIS (Table 5.5; 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.

HPRT + Px: The tubules were compactly packed and the cell height ranged from 25.6 μm in the caput to 18.9 μm in the cauda. The lumen was narrow and empty.

PLATE III

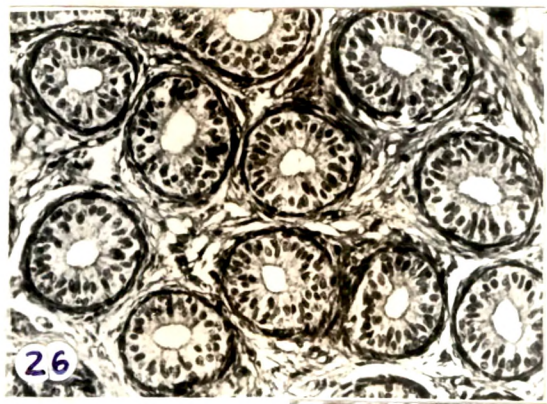
Figures 26-31: Photomicrographs of sections of epididymis of 35, 45, 60 and 90 day old neonatally hyperthyroid rats subjected to pinealectomy (HPRT + Px) [200 x].

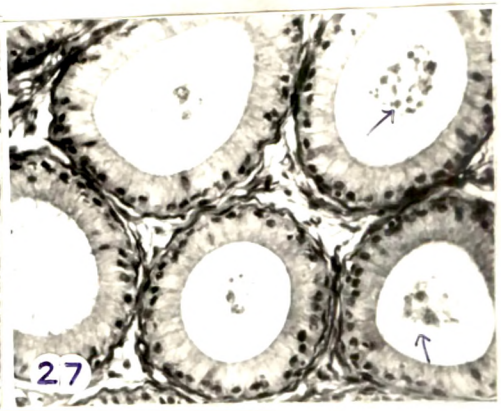
Figure 26: Section of epididymis of 35 day old rat showing compactly arranged tubules with hypertrophied epithelium and narrow lumen.

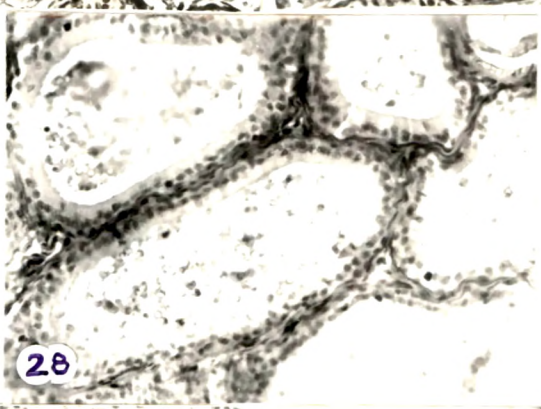
Figure 27: Section of epididymis of 45 day old rat showing large tubules with normal looking epithelial cells and containing degenerating germ cells in the lumen.

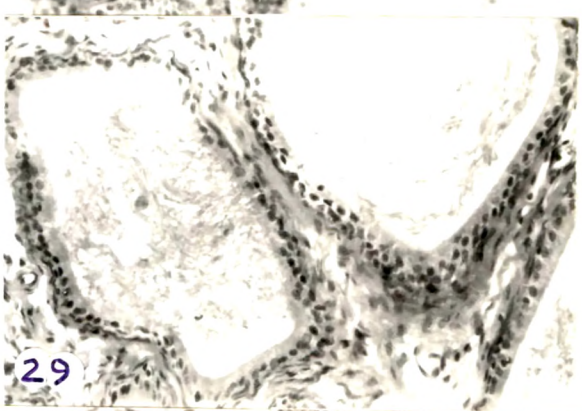
Figure 28: Section of epididymis of 60 day old rat showing normal looking compactly packed tubules containing round spermatids in the lumen.

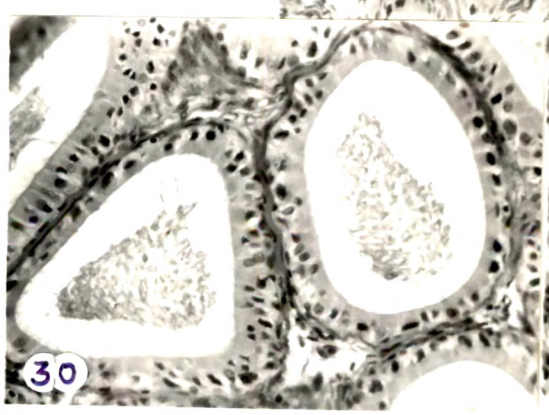
Figures 29-31: Sections of epididymis of 90 day old rat showing large tubules with normal looking epithelial cells and the lumen containing abundant sperms.

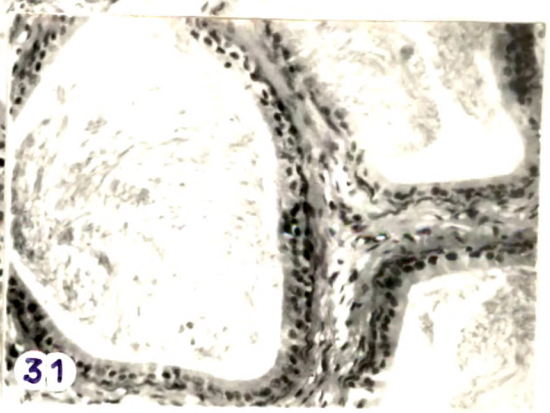

26


27


28


29


30


31

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.

HPRT + Px: Tubules were large with normal looking epithelial cells. The average cell height in the caput was 30.8 μm and in the cauda 19 μm. The lumen was full of degenerating germ cells.

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.

HPRT + Px: The tubules appeared more like the controls with an average cell height of 33.33μm in the caput and 20.8 μm in the cauda. The lumen contained many round spermatids.

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.

HPRT + Px: The tubules were large with normal looking cells and the lumen contained abundant sperms. The average cell height was almost similar in caput and cauda and measured 16.3 μm.

STRUCTURE OF SEMINAL VESICLE (Plate IV)

35 Day Old

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

HPRT + Px: The secretory epithelium was convoluted with hypertrophied cells. The lumen had little secretory material.

45 Day Old

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

HPRT + Px: The epithelium was well formed and convoluted and the cells appeared normal.

PLATE IV

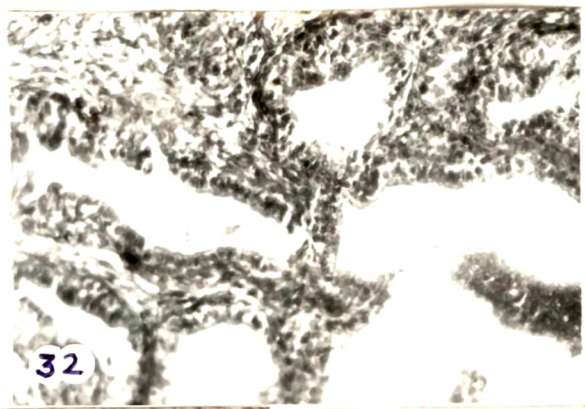
Figures 32- 36: Photomicrographs of sections of seminal vesicle of 35, 45, 60 and 90 day old neonatally hyperthyroid rats subjected to pinealectomy (HPRT + Px) [200 x].

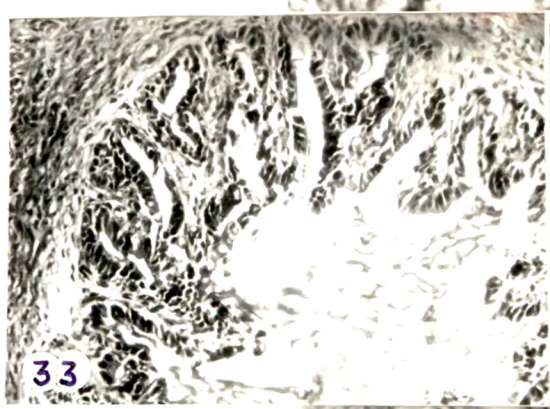
Figure 32: Sections of seminal vesicle of 35 day old rat showing convoluted secretory epithelium with hypertrophied cells and no secretory material in the lumen.

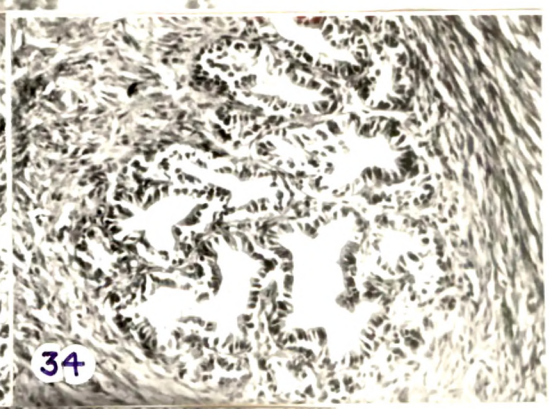
Figures 33 and 34: Sections of seminal vesicle of 45 day old rat showing well formed epithelium with normal looking cells.

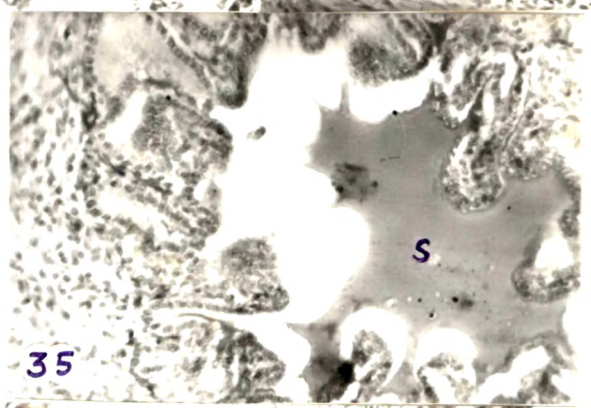
Figure 35: Section of seminal vesicle of 60 day old rat showing well formed epithelium with prominent cells. The epithelium is thickened and less convoluted and the cells were slightly hypertrophied. Secretory material (S) is present in some acini.

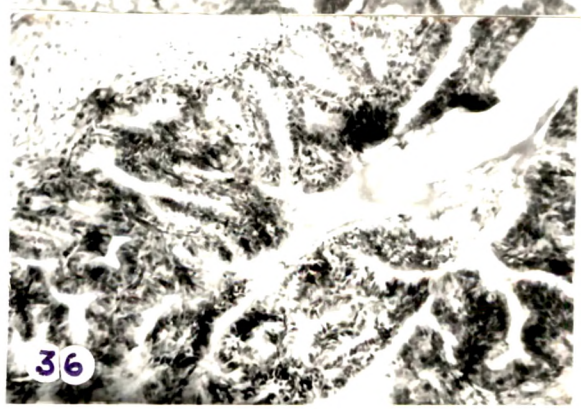
Figure 36: Section of seminal vesicle of 90 day old rat showing epithelium well formed and convoluted lined by columnar cells. The lumen contains secretory material (S).


32


33


34


S
35


36

60 Day Old

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

HPRT + Px: The epithelium was well formed with prominent cells. The epithelium appeared thicker and less convoluted. The cells were slightly hypertrophied. There was only little secretory material in the lumen.

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.

HPRT + Px: The epithelium was very prominent and convoluted lined by columnar cells. The lumen contained secretory material.

STRUCTURE OF PROSTATE (Plates V & VI)

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.

HPRT + Px: The prostatic acini were lined by low to high cuboidal epithelium. There was appreciable secretory material in the lumen.

45 Day Old

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

HPRT + Px: The acini were large and well developed lined by hypertrophied cuboidal epithelial cells. Amorphous secretory material could be seen in the lumen.

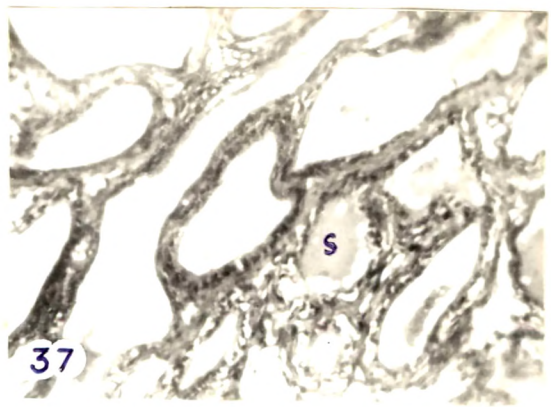
PLATE V

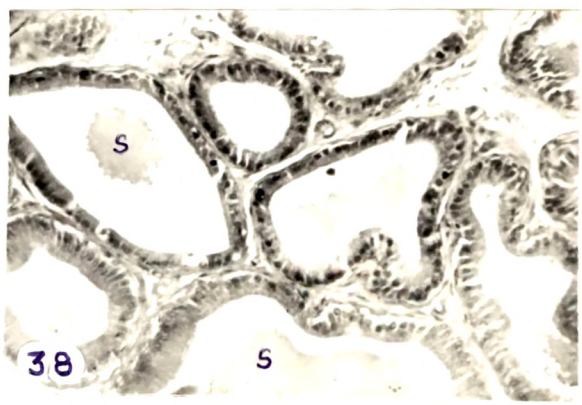
Figures 37-39: Photomicrographs of sections of prostate of 35, 45 and 60 day old neonatally hyperthyroid rats subjected to pinealectomy (HPRT + Px) [200 x].

Figure 37: Section of prostate of 35 day old rat showing prostatic acini lined by low to high cuboidal epithelium. Secretory material (S) can be seen in the lumen.

Figure 38: Section of prostate of 45 day old rat showing prostatic acini lined by large cuboidal epithelial cells. Amorphous secretory material (S) seen in the lumen.

Figure 39: Section of prostate of 60 day old rat showing well formed prostatic acini lined by cuboidal epithelium. Less secretory material (S) in the lumen.


S
37


S
38
S

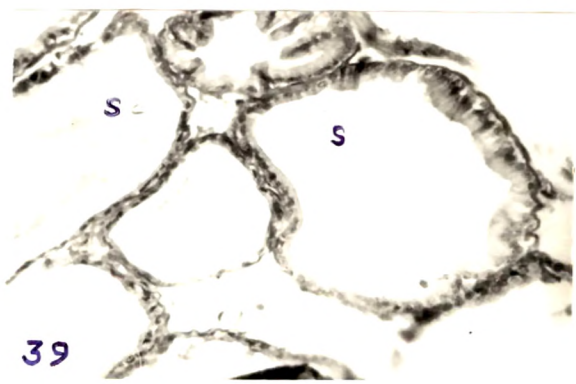
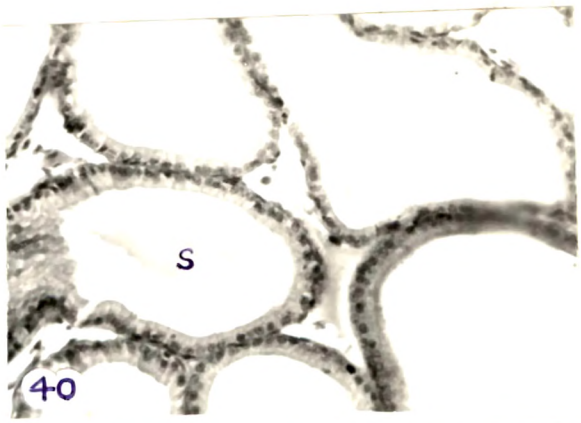
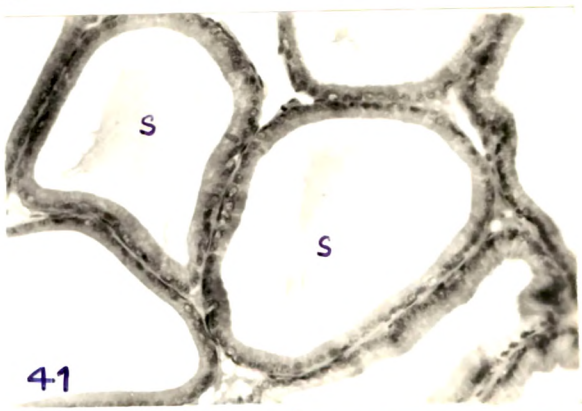

S
S
39

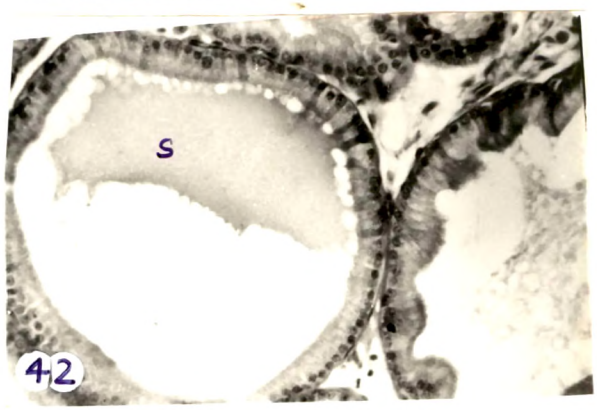
PLATE VI

Figures 40- 42: Photomicrographs of sections of prostate of 90 day old neonatally hyperthyroid rats subjected to pinealectomy (HPRT + Px) [200 x].

Figures 40- 42: Sections of prostate of 90 day old rats showing large prostatic acini lined by large cuboidal to columnar epithelial cells. Abundant secretory material (S) seen in the lumen.


S
40


S
S
41


S
42

60 Day Old

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

HPRT + Px: The acini were well formed and lined by cuboidal to columnar epithelium and had less secretory content in the lumen.

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.

HPRT + Px: The acini were large and well formed lined by large cuboidal to columnar epithelial cells. Abundant secretory material was present in the lumen.

STRUCTURE OF THYROID (Plate VII)

35 Day Old

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

HPRT + Px: The follicles were lined by hypertrophied epithelial cells due to which the lumen appeared obliterated. Some follicles contained colloid.

45 Day Old

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

HPRT + Px: Follicles were filled with colloid and were lined by cuboidal epithelium.

60 Day Old

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

HPRT + Px: The follicles were lined by hypertrophied and cuboidal epithelium. The follicles showed narrow lumen and low colloid content.

PLATE VII

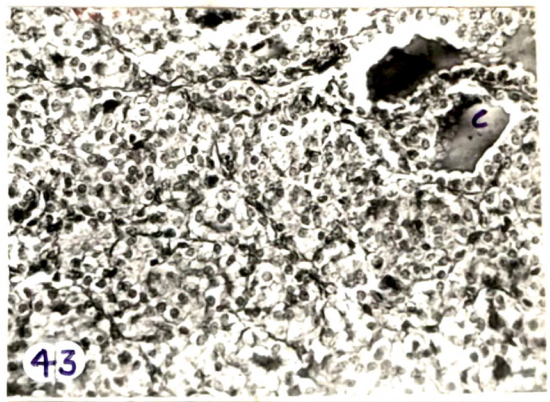
Figures 43- 47: Photomicrographs of sections of thyroid of 35, 45, 60 and 90 day old neonatally hyperthyroid rats subjected to pinealectomy (HPRT +Px) [200 x].

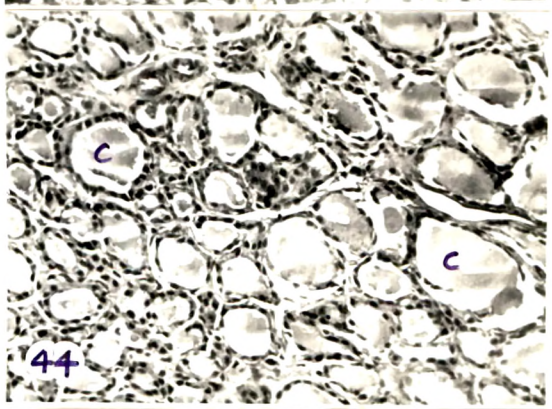
Figure 43: Section of thyroid of 35 day old rat showing follicles lined by hypertrophied cells. Lumen are obliterated and a few follicles contain colloid (C).

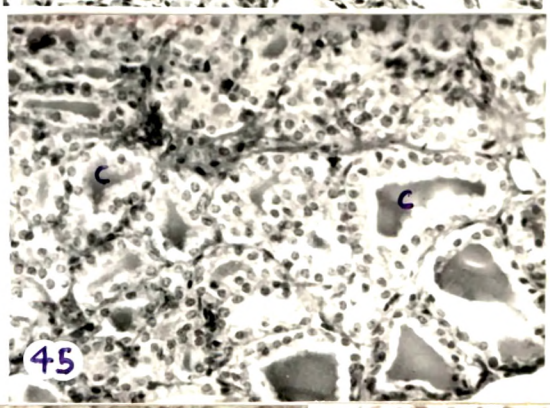
Figure 44: Section of thyroid of 45 day old rat showing follicles lined with low cuboidal epithelium, containing colloid (C).

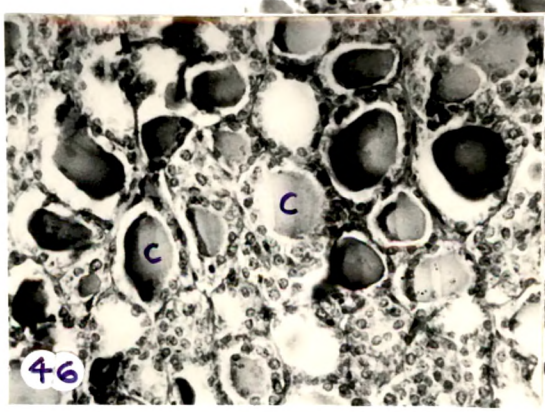
Figure 45: Section of thyroid of 60 day old rat showing follicles lined by hypertrophied epithelial cells and the follicles show narrow lumen and low colloid (C) content.

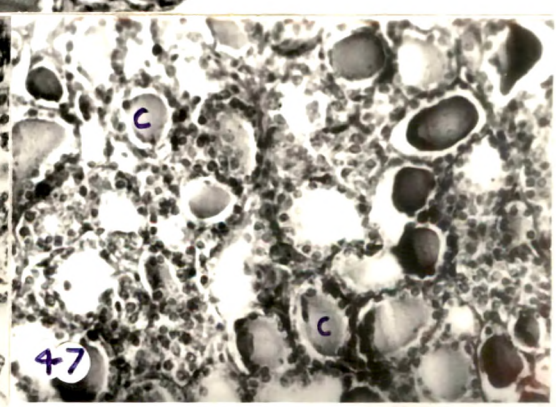
Figures 46 and 47: Sections of thyroid of 90 day old rat showing follicles lined by cuboidal epithelium and containing different degrees of colloid (C) content.


C
43


C
C
44


C
C
45


C
C
46


C
C
47

90 Day Old

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

HPRT + Px: The follicles were lined by cuboidal epithelium and showed varying degrees of colloid content ranging from high, moderate to low.

III. HISTOCHEMICAL OBSERVATIONS (Plates VIII & IX)

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 in control rats. The 3α-HSDH activity was weakly localised in the tubules but not in the Leydig cells of control rats. In the HPRT + Px rats, appreciable 3ß-HSDH activity was discernible in the interstitium while it was weak in the tubules. The activity with DHEA as the substrate was prominent than with P . 17ß-HSDH activity was strong in the tubules and weak in the interstitium. Activity of 3α-HSDH was prominent in the interstitium while the tubules showed no activity.

45 Day Old

In the control rats, mild activity could be seen in the Leydig cells, noticeably more with DHEA as the substrate. The 3ß-HSDH was reduced as compared to 35 day's. There was mild enzyme activity in the Leydig cells with DHEA as the substrate which was not evident with P. In the control animals, the Leydig cells were weakly 17ß-HSDH active while the tubules showed significant activity. Though the enzyme activity was localised uniformly within the tubules containing early stages of germ cells, the enzyme activity was localised more in the luminal part in 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 rats. In the HPRT + Px rats the activity of 3ß-HSDH was mild in the interstitium and weak in the tubules. The activity of

PLATE VIII

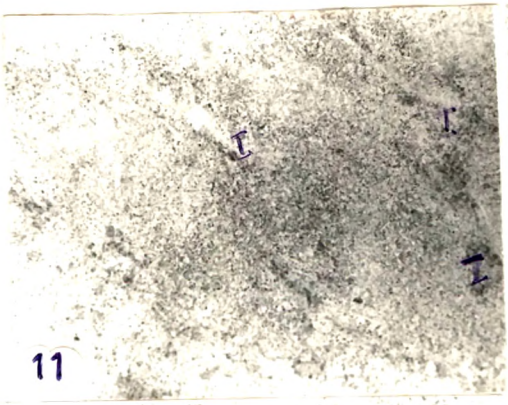
Figures 11-18: Photomicrographs of sections of testis of 35, 45, 60 and 90 day old neonatally hyperthyroid rats subjected to pinealectomy showing histochemical localisation of 3α and 17ß HSDH (65 x).

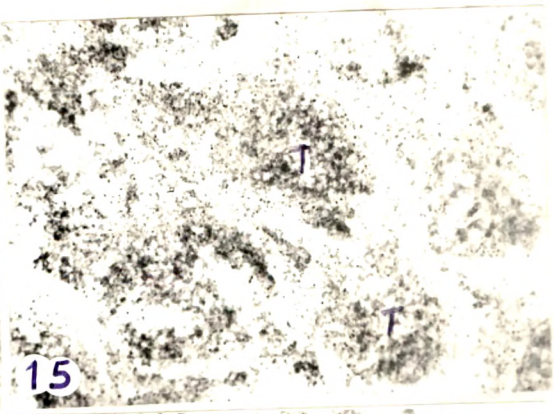
Figures 11 and 15: Sections of testis of 35 day old rats showing prominent activity of 3α HSDH (Fig. 11) in the interstitium (I) and that of 17ß HSDH (Fig. 15) in the tubules (T).

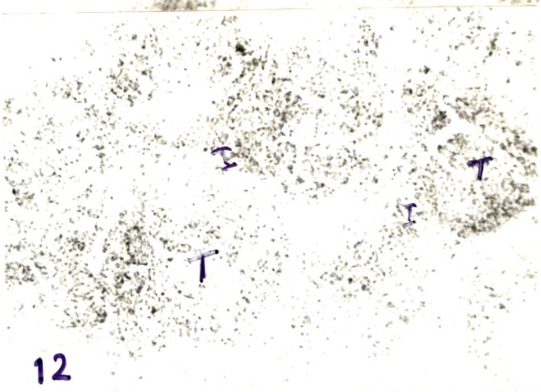
Figures 12 and 16: Sections of testis of 45 day old rat showing weak activity of 3α HSDH (Fig 12) in both tubules (T) and interstitium (I) and strong activity in the tubules (T) and mild activity in the interstitium (I) of 17ß HSDH (Fig.16).

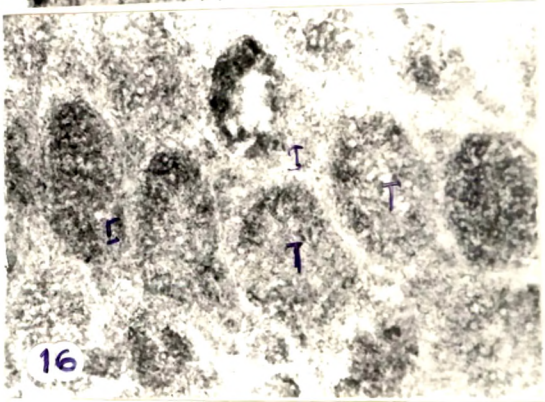
Figures 13 and 17: Sections of testis of 60 day old rats showing mild activity in the interstitium (I) and noticeable activity in the tubules (T) for 3α HSDH (Fig. 13) and mild activity in the interstitium (I) and strong activity in the tubules (T) for 17ß HSDH (Fig.17).

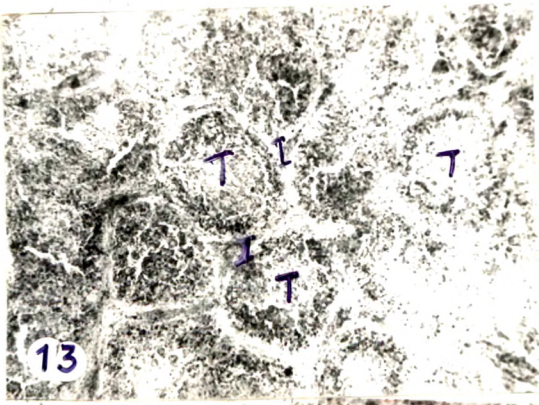
Figures 14 and 18: Sections of testis of 90 day old rats showing noticeable to mild activity in the interstitium (I) and intense activity in the tubules (T) containing advanced stages of germ cells for both 3α HSDH (Fig.14) and 17ß HSDH (Fig.18).

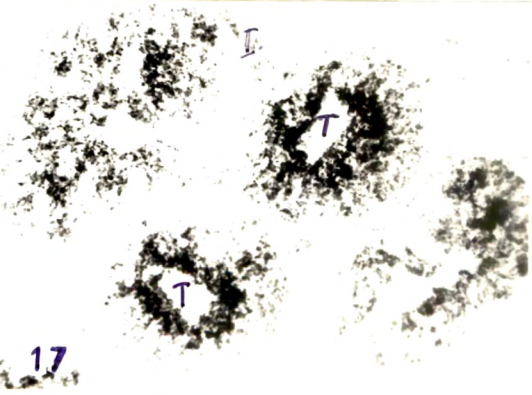

I
I
I
11

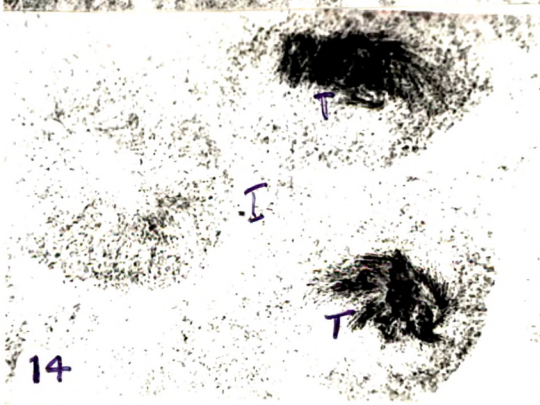

T
T
15


I
T
I
T
12


I
T
I
T
16


T
I
T
I
T
13


I
T
T
17


T
I
T
14

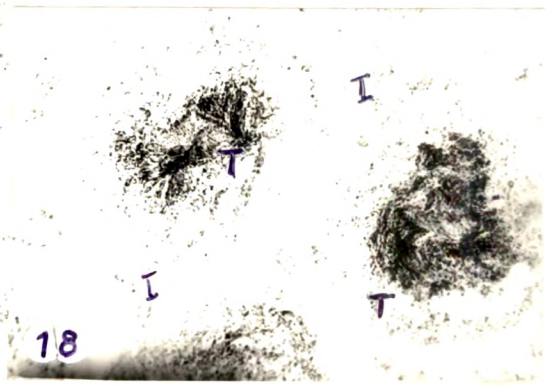

I
T
I
T
18

PLATE IX

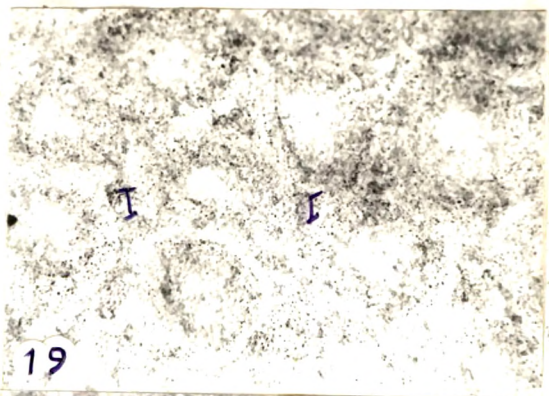
Figures 19- 25: Photomicrographs of sections of testis of 35, 45, 60 and 90 day old neonatally hyperthyroid rats subjected to pinealectomy (HPRT +Px) showing histochemical localisation of 3ß HSDH using P and DHEA as substrates (65 x).

Figures 19 and 22: Sections of testis of 35 day old rat showing appreciable activity of 3ß HSDH in the interstitium (I).

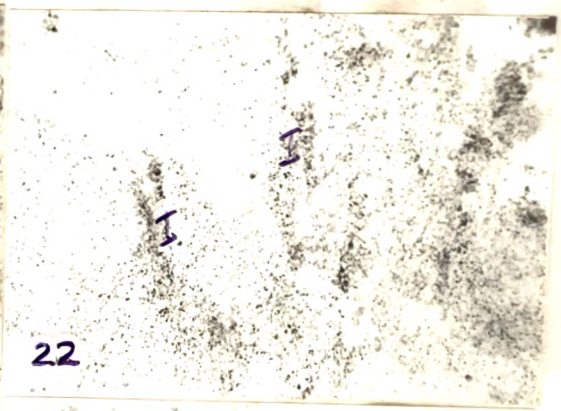
Figures 20 and 23: Sections of testis of 45 day old rats showing noticeable enzyme activity in both interstitium (I) and tubules (T) with P (Fig. 20) and low activity in the interstitium (I) with DHEA (Fig. 23).

Figure 24: Section of testis of 60 day old rat showing noticeable activity in the interstitium (I) with DHEA as the substrate.

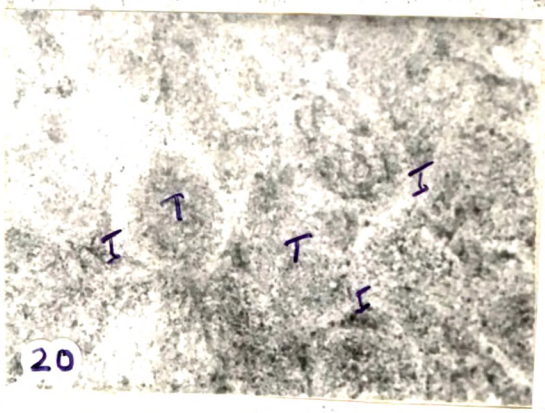
Figures 21 and 25: Sections of testis of 90 day old rat showing mild activity in the interstitium (I) and strong activity in the tubules (T) containing advanced stages of germ cells. More pronounced with DHEA as the substrate (Fig. 25).



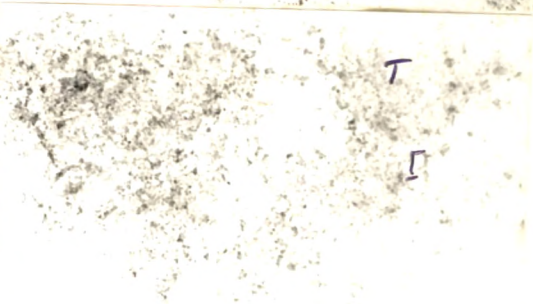
19



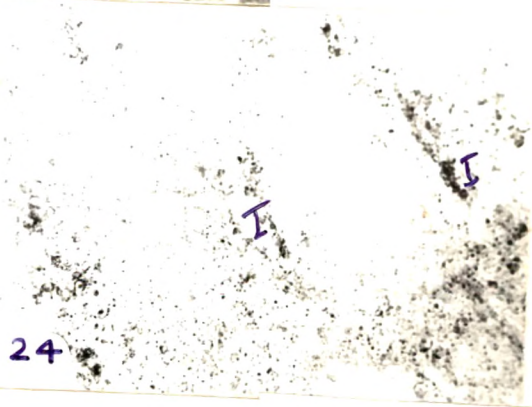
22



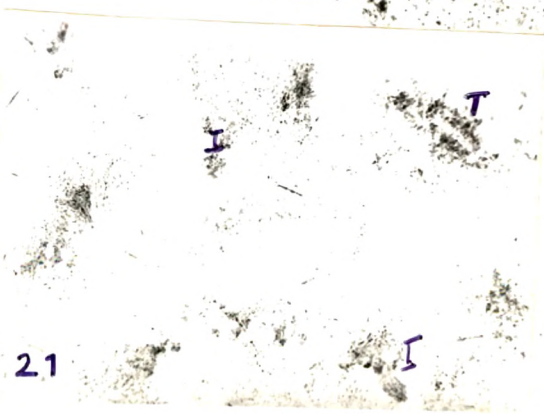
20



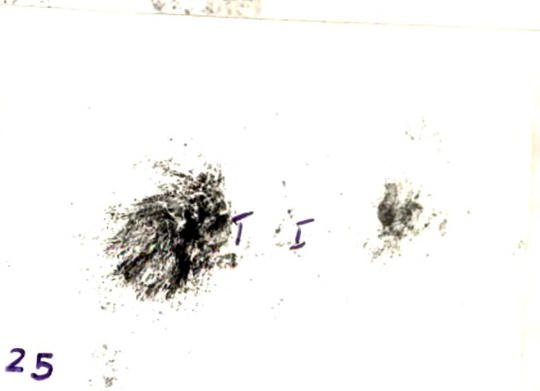
23



24



21



25

17ß-HSDH was weak in the interstitium and strong in the tubules. There was generally no activity of 3α-HSDH in the interstitium except for a weak response by a few cells. The activity of the enzyme in the tubules was also very weak.

60 Day Old

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 in control animals. Relatively the enzyme activity was weak with P. Compared to 45 days, the enzyme activity was significantly more. 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. In the HPRT + Px rats, the appreciable activity of 3ß-HSDH could be seen in the interstitium, more with DHEA as the substrate while there was very weak activity in the tubules. The activity of 17ß-HSDH was appreciable in the interstitium and mild in the tubules. The interstitium showed mild activity of 3α-HSDH while some tubules showed moderate and some others mild activity.

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 rats. The interstitial cells were mildly enzyme responsive. Relatively, the enzyme activity was more than that of 3ß-HSDH. In the control rats, 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. In the HPRT + Px rats, the activity of 3ß-HSDH was mild in the interstitium and in the tubules. However, tubules with advanced stages of germ cells showed strong localization. The tubules showed intense response for 17ß-HSDH with the localization being more prominent towards the center of the tubules containing advanced stages of germ cells. The interstitium depicted mild localization. Though the activity of 3α-HSDH was mildly localized in the interstitium, the tubules containing advanced stages of germ cells showed strong localization in the luminal part.

IV. SERUM HORMONE PROFILE (Table 5.6; 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 35 day levels of T_3 and T_4 in the HPRT + Px rats were significantly lower compared to the controls. Thereafter, their levels increased to attain control levels. Whereas the serum T_4 attained its highest level by 45 days, that of serum T_3 attained by 60 days. The hormone levels remained more or less in the same range thereafter.

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 HPRT + Px rats had significantly higher T levels at 35 days. The hormone level remained more or less steady thereafter, though slightly decreased, till 60 days and then attained a very high level at 90 days.

DISCUSSION

The present results show that the HPRT + Px animals have reduced body weight and growth rate during the entire period of study. Compared to the controls, the growth kinetics revealed a

Table 5.6 Chronological alterations in Serum Triiodothyronine, Thyroxine and Testosterone levels in intact and hyperthyroid rats subjected to pinealectomy (HPRT + 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
HPRT + Px	2.02 ± 0.28^{a}	1.99 ± 0.16^{d}	2.40 ± 0.22^{d}	2.42 ± 0.13^{ns}	39.69 ± 2.44^{d}	64.05 ± 3.81^{d}	62.53 ± 5.16^{d}	60.68 ± 3.12^{c}	1.26 ± 0.22^{d}	0.98 ± 0.20^{d}	1.03 ± 0.17^{c}	2.25 ± 0.19^{c}

@ Values expressed as Mean ± SD of five experiments

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

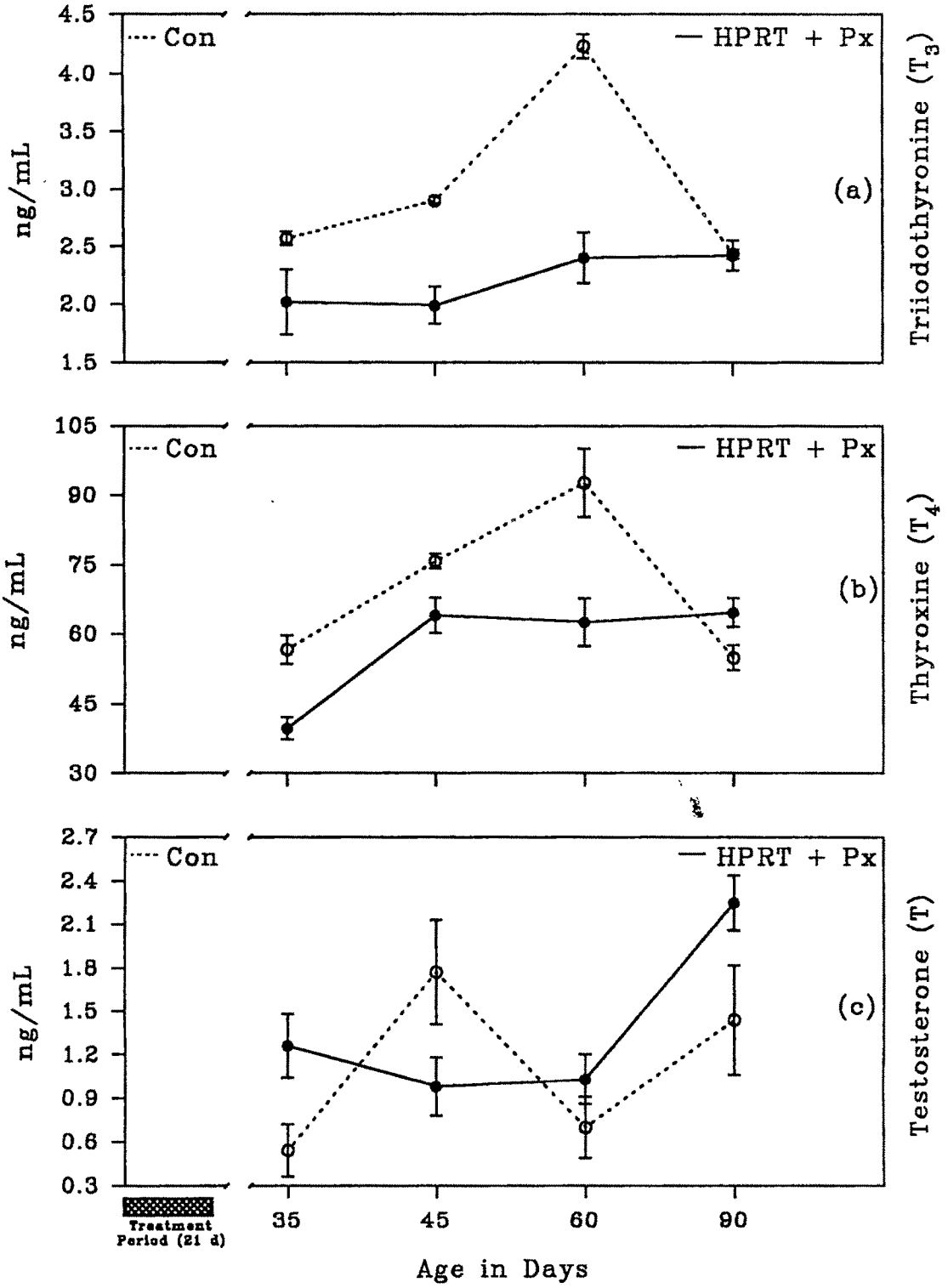


Fig. 12 (a, b & c) Chronological alterations in serum T_3, T_4 and T levels in hyperthyroid rats subjected to pinealectomy (HPRT + Px)

lowered threshold of growth rate though less significant, this is similar to what was observed previously in hyperthyroidic rats (chapter 4). This could be attributed to the reduced GH secretion due to early neonatal hyperthyroidism as envisaged earlier in hyperthyroid rats (chapter 4). Evidences are available to show that altered thyroid hormone status in the immature stages can reduce GH secretion (see, Giustina and Wehrenberg, 1995). Similar reduction in body weight due to neonatal T_3 administration has also been considered possibly due to reduced GH levels (Van Haaster *et al.*, 1993). The persisting reduced growth kinetics even after cessation of T_4 treatment indicates lowering of the set point of the hypothalamic-pituitary unit growth regulating the GH secretion. This effect of thyroid hormone on the growth hormone axis appears to be manifested in the immediate neonatal period itself. This conclusion is drawn based on the fact that the HPRT + Px rats remains hyperthyroidic only till the duly expected establishment of pineal functions (from 10 day onwards) as neonatally Px animals become hypothyroidic in the juvenile period (to be discussed later). Apparently, it is during those first 10 days of hyperthyroidic state, the set-point of GH secretion is lowered and remains imprinted.

In the earlier study on hyperthyroidism, the testes weight was significantly reduced at 90 days and was only 67% of the controls. In contrast to that in the present study, Px nullified the hyperthyroidic effect and the paired testes weight at 90 days was the same as that of the controls. However, the testes weight was less than the controls in the early periods. This could be due to the generalised growth retardation caused by reduced GH secretion as inferred earlier. FSH which controls gonadal growth generally starts increasing after 10 days from the low neonatal level. In the earlier study it was shown that neonatal hyperthyroidism decreases gonadotropin levels (chapter 4), while neonatal Px increases the levels of gonadotropins (chapter 2). Since, the pineal matures and starts its functions in rats only between 10 to 15 days, the Px induced effect on gonadotropins can apparently be manifested only after that period. In this context, the initial retardation in the growth of testes seems to be due to the reduced FSH level, which is clearly the hyperthyroidic effect. Due to this early effect of hyperthyroidism, the increase

in gonadotropin levels, a consequence of Px, is attenuated, as compared to the pinealectomised animals (chapter 2). Another effect of Px is its lowering influence on thyroid hormones as inferred earlier (chapter 2). Apparently, in the period between 25 and 35 days, there is a hypothyroidic state despite T_4 administration, as the dosage administered is so low that it is unable to offset the effect of Px. The concurrent increased FSH level seems to stimulate the growth of testes by inducing spermatogonial and Sertoli cell proliferation. Though some studies on other strains of rat have shown that the thyroid hormone inhibits Sertoli cell proliferation and induces Sertoli cell differentiation (Cooke and Meisami, 1991; Cooke *et al.*, 1992; Kirby *et al.*, 1992; Van Haaster *et al.*, 1992; Hess *et al.*, 1993), in the Charles-foster strain, thyroid hormone was shown to be less important on its influence on Sertoli cell proliferation though, it has a permissive influence in inducing differentiation (chapter 1). From the above studies on Charles-foster strain, it was inferred that FSH alone is responsible for Sertoli cell proliferation though it is dependent on thyroid hormone for controlling Sertoli cell differentiation. In the present study, it is evident that the increased FSH level induces Sertoli cell proliferation which is evident from the increased tubular diameter at 35 days. The increase in LH level caused by Px is also reflected in the histochemically observed weak localization of 17ß-HSDH in the interstitium, not observable in the controls.

Since the thyroid hormone level increases to the optimal levels only by 45 days, Sertoli cell differentiation is delayed, as was the case in the previous study involving Px (chapter 2). This, though prolongs the period of Sertoli cell proliferation, is nevertheless not conducive for the survival of the advanced stages of germ cells as, they are depended on structurally and functionally differentiated Sertoli cells (O' Donnell *et al.*, 1995; McLachlan *et al.*, 1996; chapter 1). This is very obvious in the present study as there was rampant degeneration of germ cells and decrease in testes weight at 45 days. The increased germ cell degeneration is corroborated by the increased histochemical localization of 17ß-and 3α-HSDH in the tubules, an observation which was seen even in the earlier studies (chapters 1-4). The delayed Sertoli cell differentiation

ultimately results in reestablishment of spermatogenesis as evident from the histological appearance and the noted presence of round spermatids. From 60 day onwards the testicular functions seems to be greatly hastened as almost all the tubules contained spermatozoa. The increased Sertoli cell proliferation was due to a delay in their differentiation is clearly evident from the steady growth rate of testes from 35 to 90 days, as well as the increase in tubular diameter between 60 and 90 days and, the histologically observable greater number of germ cells compared to the controls at 90 days. Though the testes weight at 90 days was less than that of the Px animals, it is likely that further growth after 90 days can result in hypertrophid testes. This can be speculated from the recorded growth rates of testes which has remained steadily high from 45 day onwards. Apparently, Px alone or a combination of neonatal hyperthyroidism and Px can increase sperm production in the Charles-foster strain of rat. Though both could increase germ cell production, HPRT + Px seems to have a qualitatively better influence.

The morphometric and histological observations reveal an initial growth retardation in the epididymis of HPRT + Px animals as seen at 35 days. The reduced tubular diameter with hypertrophid cells and narrow lumen bear testimony to this. However, both the seminal vesicle and prostate weighed as much as those of controls and, in fact the initial weight of prostate at 35 days was significantly greater. At 90 days while the prostate weight equalled to that of the controls, the seminal vesicle weighed significantly greater and the epididymis significantly lower. These differences are suggestive of differential hormonal requirement/sensitivity in controlling their growth. In the earlier studies it was shown that a hypothyroidic state in the juvenile and prepubertal periods in pineal intact animals is more detrimental to the growth of accessory organs due to the potentiated growth retardatory influence of melatonin (chapter 1). This was further confirmed by a similar observation of lowered accessory organ weights in rats subjected to neonatal hyperthyroidism. This was again accredited to the potentiated growth retarding influence of melatonin in a lowered serum thyroid hormone state, as withdrawal of T_4 was immediately followed by a hypothyroidic state (chapter 4). The present observations on the

weight and structure of the prostate and seminal vesicles indicate that the absence of melatonin despite the hypothyroid state favours normal growth of these organs. The increased growth rates seen between 45 and 90 days in the case of seminal vesicles and between 60 and 90 days in the case of prostate are related with the synergistic growth and maturation promoting influence of PRL and T. The delay in the final growth and maturation seen in the case of prostate can be related to the subnormal PRL levels induced by Px in the earlier periods (chapter 2). The seminal vesicle not exposed to the growth retardatory influence of melatonin in the earlier periods, seems to show a hypersensitive response towards the synergistic action of PRL and T as seen in the present study.

In an earlier study it was inferred that neonatal hyperthyroidism decreases GH secretion and, based on the observations of body weight in the present study, it was inferred earlier that hyperthyroidism in the immediate neonatal days (up to 10 days) permanently lowered the set-point for GH secretion. The consistently reduced weight of the epididymis seen in the present study probably suggests a relatively greater dependence of this organ on GH for its growth. A similar observation of reduced epididymal weight in rats rendered neonatally hyperthyroidic (chapter 4) strengthens the present contention. Interestingly the diameter of the epididymal tubules is significantly greater in the HPRT + Px animals at 90 days. In this context it may be speculated that the epididymis in these experimental animals might have reduced length compensated by increased diameter.

Finally, it can be concluded from the present results that transient neonatal hyperthyroidism in pinealectomised rats has a favourable influence on Sertoli cell proliferation and increased germ cell production. Neonatal hyperthyroidism seems to lower the hypothalamic set-point regulating GH secretion. The consequent lowered levels of GH has a persistent influence on body and epididymal growth. The present results also emphasize a definite growth retardatory influence of melatonin on sex accessory organs in the immature stages and the role of thyroid hormones in resisting the same.