CHAPTER 4

TRANSIENT NEONATAL HYPERTHYROIDISM DECREASES ADULT TESTIS SIZE AND ACCESSORY ORGAN WEIGHTS IN THE RAT: CONSEQUENCE OF ALTERED GROWTH AND THYROID HORMONE SET POINTS AND GONADOTROPIN LEVELS

The importance of thyroid hormones for normal growth and development of almost all organ systems is well recognised in mammals. A clear relationship between thyroid activity and the development of the male reproductive system has been documented (Longscope, 1976). It has been shown that prepubertal development of gametogenic cells is dependent on thyroid activity (Chowdhury and Arora, 1984). Though there are reports on unresponsiveness of the adult testis to triiodothyronine (Barker and Klitgaard, 1962; Oppenheimer *et al.*, 1974), some of the recent studies provide evidence for a possible influence of thyroid hormone on testicular functions (Palmero *et al.*, 1990; 1992). Both *in vivo* and *in vitro* experiments have demonstrated the favorable influence of the thyroid hormone in the early postnatal development of rat testis. It has been shown that *in vivo* administration of triiodothyronine for three days during the first postnatal week causes a 60% increase in testis size (Chowdhury *et al.*, 1984; Jannini *et al.*, 1993; Van Haaster *et al.*, 1993). Similarly, neonatal testis fragments cultured *in vitro* in presence of thyroid hormone depicted a significant increase in the size of seminiferous cords and in the number of goanocytes along with decreased percentage of degenerating germ cells (Bourget *et al.*, 1987). A stimulatory effect on seminiferous epithelium has been documented by injections of thyroxine

in the prepubertal period (Amin and El-Sheikh, 1977). Longer *in vivo* exposure to triiodothyronine for 60 days neonatally has also been shown to accelerate testis development (Dacosta and Carlson, 1933; Amin and El-Seikh, 1977). In contrast to the above reports of favorable influence of thyroid hormones on testis, recent studies have shown hyperplasia of testicular cells under neonatal hypothyroidism resulting in a greatly hypertrophied testis in the adult condition (Cooke *et al.*, 1991; Meisami *et al.*, 1992; Kirby *et al.*, 1992; Hess *et al.*, 1993). However, similar experiments carried out in Charles foster strain failed to reproduce these effects (chapter 1) and was attributed to a possible strain difference. Corollary experiments involving neonatal hyperthyroidism showed reduced Sertoli cell proliferation, accelerated tubular lumen formation and reduced testis size (Van Haaster *et al.*, 1993). Since, neonatal hypothyroidism elicited a differential response in the Charles foster strain, it was natural that the response of this strain of rats to neonatal hyperthyroidism need to be studied. The present study in this context deals with the influence of induced transient neonatal hyperthyroidism on the developmental profile of the male reproductive system.

RESULTS

I. MORPHOMETRIC OBSERVATIONS

BODY WEIGHT (Table 4.1; Fig. 1 & 2)

The HPRT animals showed significantly lesser body weight compared to controls at 35 days. Whereas the control animals showed a decrement in body weight at 45 days, the HPRT animals showed continuous increment in body weight without decrease at any stage. The body weight at 90 days was higher in control animals. The control animals showed maximum percentage increment in body weight between 45 and 60 days and the HPRT animals showed between 60 and 90 days. The growth rate of control animals was maximum between 45 and 60 days and that of HPRT animals between 0 and 35 days.

Table 4.1 Chronological alterations in Body Weight (gm), Percentage Difference and Per Day Growth Rate in intact and hyperthyroid (HPRT) rats

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	36 00 ± 3.00^{d}	50.00 ± 5.09^{ns}	58.64 ± 5.93^{d}	84.80 ± 8.19^{d}	+ 38.89	+ 17.28	+ 44.61	+ 135.56	1.03 ± 0.09^{a}	1.40 ± 0.10^{d}	0.58 ± 0.04^{d}	0 87 ± 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

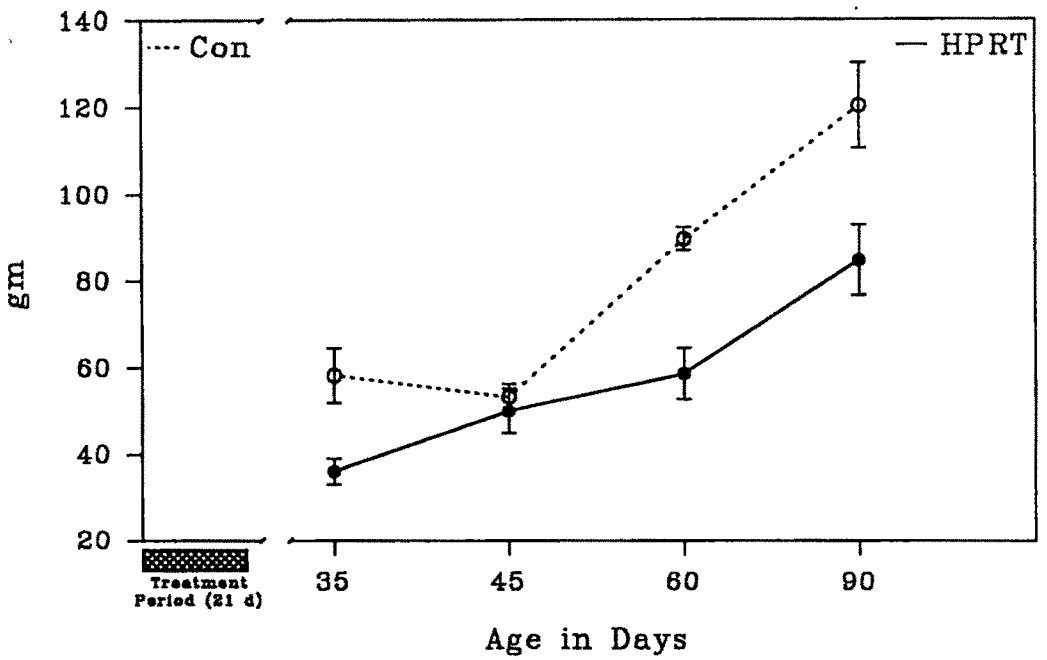

Fig. 1 Chronological alterations in body weight of neonatal rats subjected to transient hyperthyroidism (HPRT)

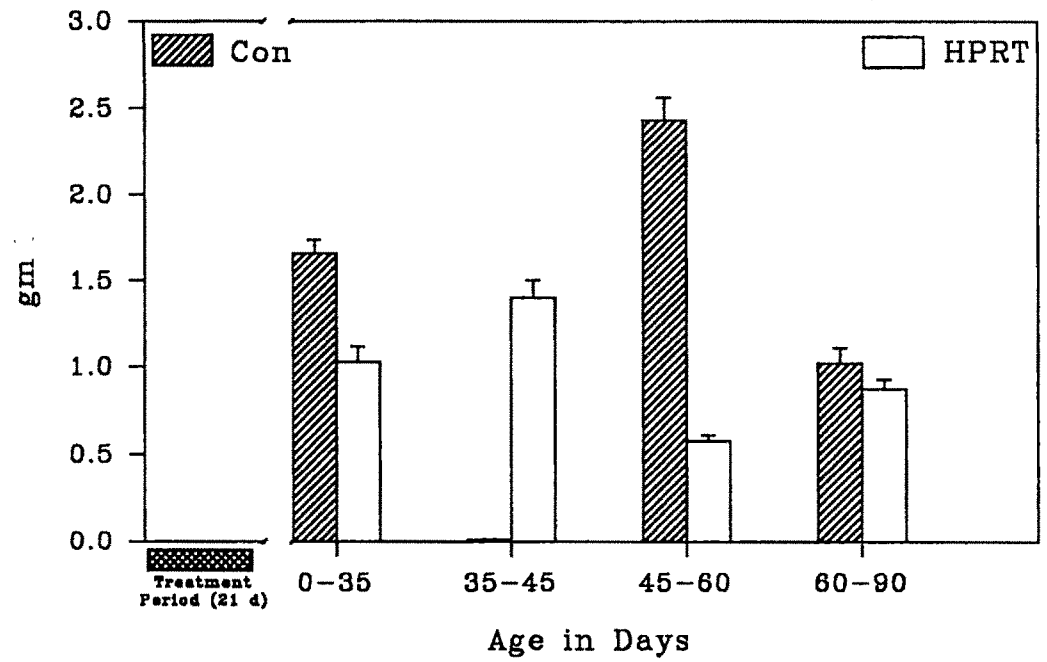

Fig. 2 Per day body growth in intact and hyperthyroid rats

ORGAN WEIGHTS

Testes (Table 4.2 a, b; Fig. 3 a, b & 4)
At 35 days, the testes weight was significantly higher in control animals compared to HPRT animals. The testes weight increased continuously from 35 to 90 days in both the groups. The final testes weight was significantly lowered in HPRT rats at 90 days compared to controls. On a percentage basis, the maximum growth in control animals occurred between 45 and 60 days, the same occurred in HPRT animals between 35 and 45 days. Both the groups of rats showed continuous increase in relative weight from 35 to 90 days. The control animals depicted maximum growth rate between 45 and 60 days, while the same was shown between 35 and 45 days in HPRT rats.

Epididymis (Table 4.2 a, b; Fig. 5 a, b & 6)
The absolute weight of epididymis was significantly lower in HPRT group of rats as compared to the controls at 35 days. Whereas the control animals showed a transient decrement in weight at 45 days, while the HPRT animals recorded continuous increment. At 90 days the weight of the epididymis was significantly less than the controls in HPRT rats. The maximum increase in relative weight was between 45 and 60 days in the control animals whereas the HPRT animals showed the same between 60 and 90 days. The maximum percentage growth of epididymis in control animals occurred between 45 and 60 days, while the same occurred between 60 and 90 days in HPRT animals. The growth rate of epididymis in both control and HPRT groups of rats reflected the changes in percentage growth.

Seminal Vesicle (Table 4.3 a, b; Fig. 7 a, b & 8)
The weight of seminal vesicle was significantly lower in HPRT rats compared to the controls at 35 days. Though the control animals showed a decrement in between at 45 days before recording continuous increment, the HPRT animals showed continuous increase. At 90 days the final weight of seminal vesicle was significantly reduced in the HPRT rats compared to the controls. The percentage increment in the weight of seminal vesicle was the greatest between

Table 4.2 (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 (HPRT) rats

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	320.62^{d} ± 38.80	598.37^{d} ± 45.85	928.50^{d} ± 96.52	1624.41^{d} ± 107.43	0.89^{ns} ± 0.08	1.21^{a} ± 0.13	1.59^{ns} ± 0.25	1.92^{c} ± 0.07
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	42.87^{d} ± 6.56	73.78^{c} ± 5.87	94.28^{d} ± 11.69	395.04^{d} ± 26.36	0.12^{d} ± 0.01	0.15^{c} ± 0.02	0.16^{d} ± 0 02	0.47^{c} ± 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
	HPRT	+ 86.63	+ 55.16	+ 74.95	+ 406.65	9.16^{d} ± 0.99	27.77^{ns} ± 4.56	22.01^{d} ± 4.20	23.19^{d} ± 3.95
EPIDIDYMIS	Con	- 47.68	+ 513.56	+ 27.75	+ 310.09	3.51 ± 0.32	-	22.06 ± 6.45	3.65 ± 0.24
	HPRT	+ 72.10	+ 27.78	+ 319.01	+ 821.48	1.22^{d} ± 0.09	3.09^{d} ± 0.11	1.37^{d} ± 0.09	10.02^{d} ± 1.05

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

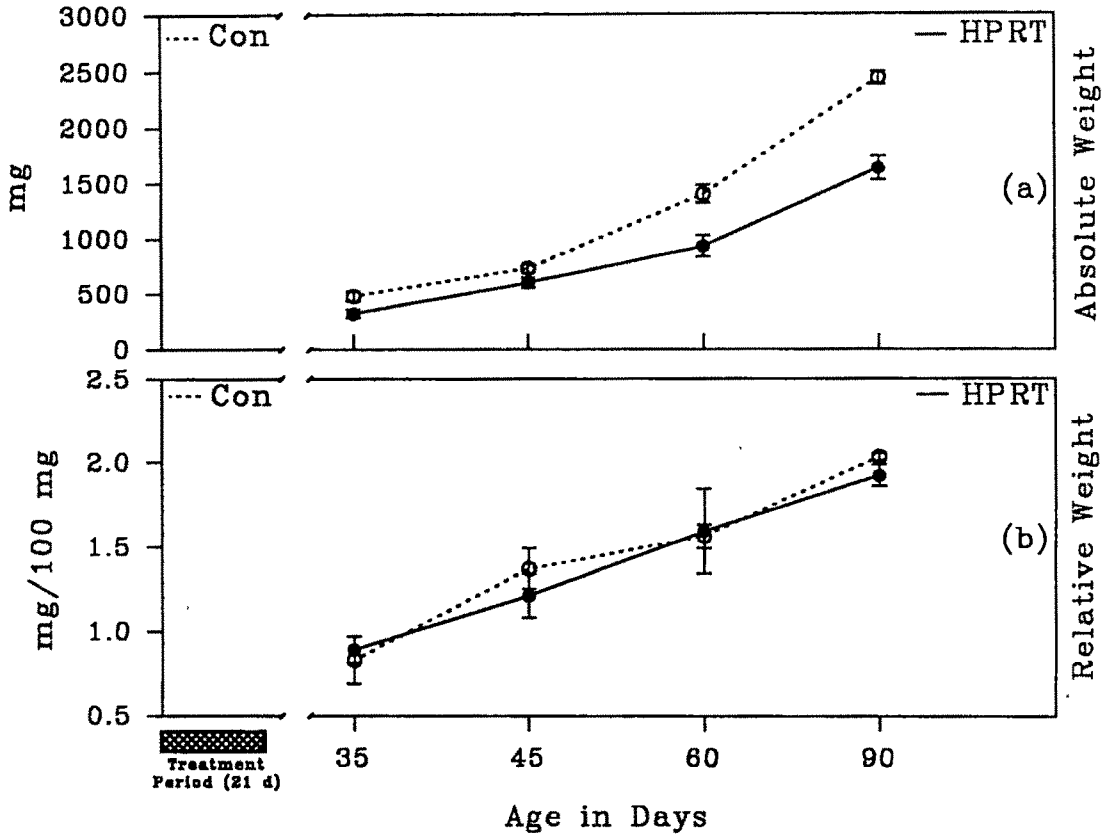

Fig. 3 (a&b) Chronological alterations in absolute and relative weights of testes in intact and hyperthyroid (HPRT) rats

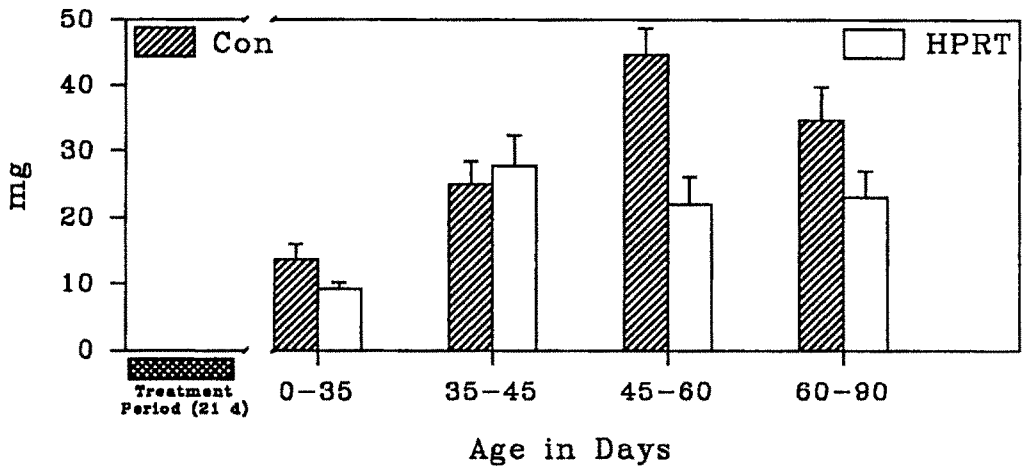

Fig. 4 Rate of growth of testes in intact and hyperthyroid rats

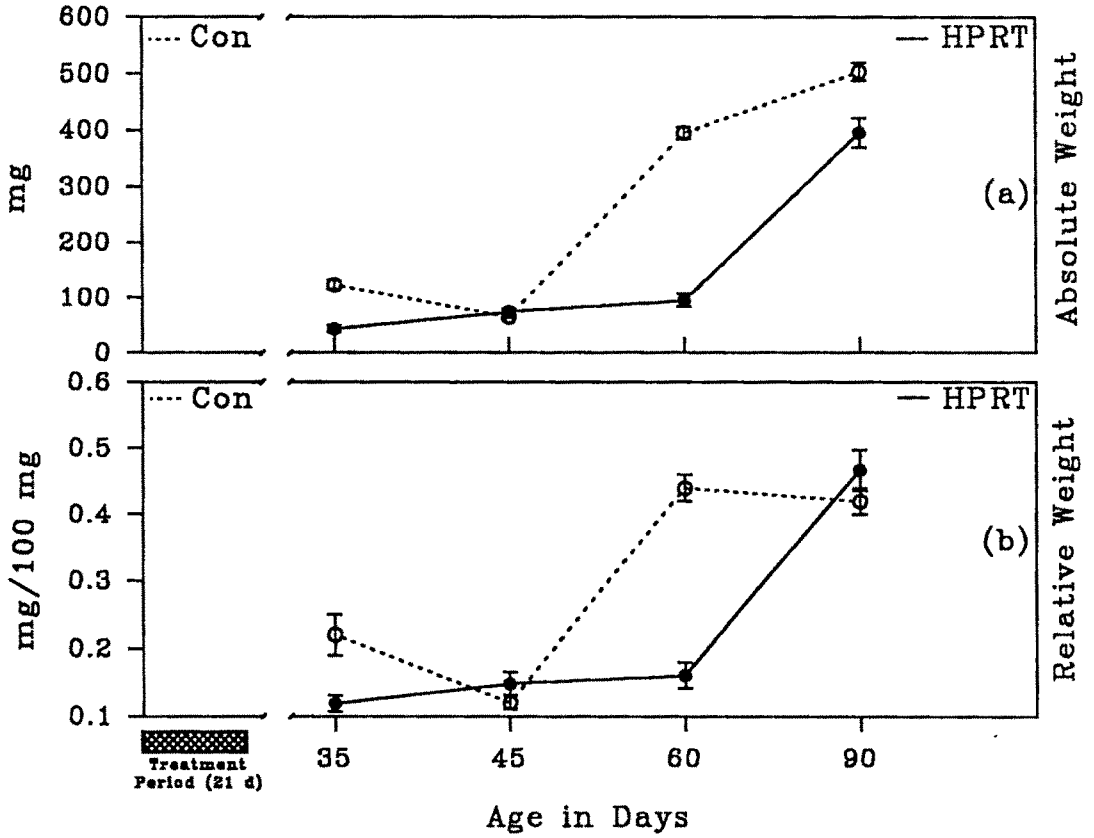

Fig. 5 (a&b) Chronological alterations in absolute and relative weights of epididymis in intact and hyperthyroid (HPRT) rats

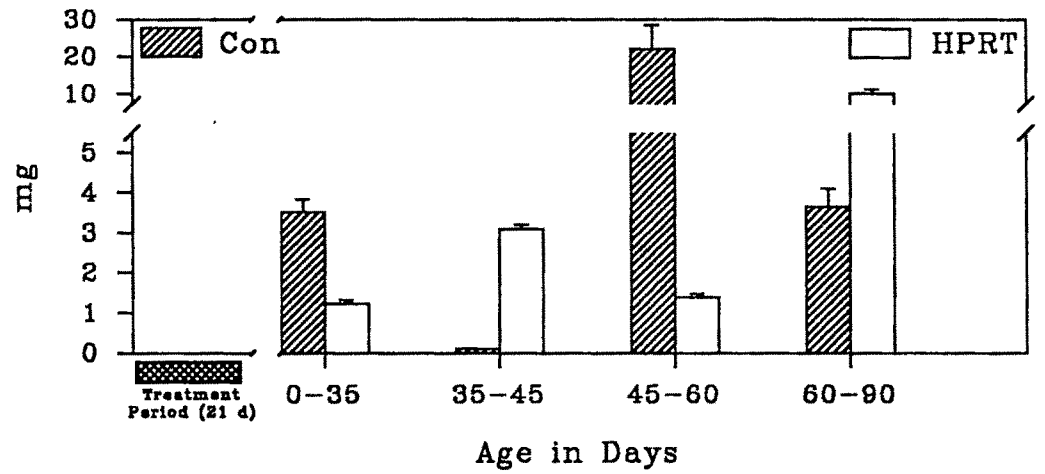

Fig. 6 Rate of growth of epididymis in intact and hyperthyroid rats

Table 4.3 (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 (HPRT) rats

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	19 40^{bd} ± 1.77	26.44^{c} ± 2.54	39.34^{d} ± 6.20	229.47^{d} ± 13.12	0.053^{c} ± 0.003	0.053^{d} ± 0.007	0.066^{ns} ± 0.009	0.271^{d} ± 0.015
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	22.88^{ns} ± 2.89	25.88^{d} ± 3.70	36.42^{d} ± 5.01	92.43^{d} ± 8.29	0.063^{d} ± 0.006	0.052^{d} ± 0.01	0.062^{d} ± 0.004	0.110^{c} ± 0.01

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	+ 36.29	+ 48.79	+ 483.29	+ 1082.84	0.55^{b} ± 0.04	0.70^{d} ± 0.01	0.86^{d} ± 0.05	6.34^{d} ± 0.39
PROSTATE GLAND	Con	- 37.94	+ 435.51	+ 89.87	+ 531 03	0.69 ± 0.09	-	4.41 ± 0.95	2.43 ± 0.89
	HPRT	+ 13.11	+ 40 73	+ 154.34	+ 304.85	0.65^{ns} ± 0.02	0 30^{d} ± 0 01	0.70^{d} ± 0.025	1 87^{d} ± 0.58

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

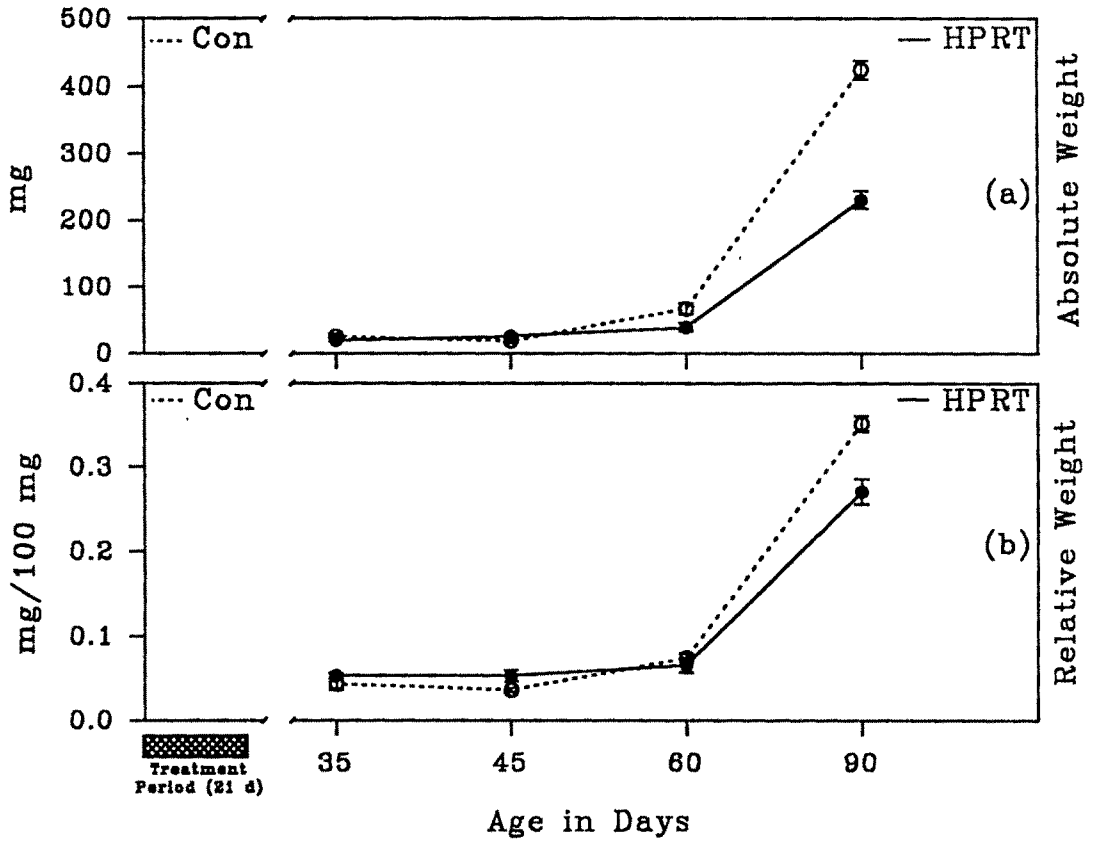

Fig. 7 (a&b) Chronological alterations in absolute and relative weights of seminal vesicle in intact and hyperthyroid (HPRT) rats

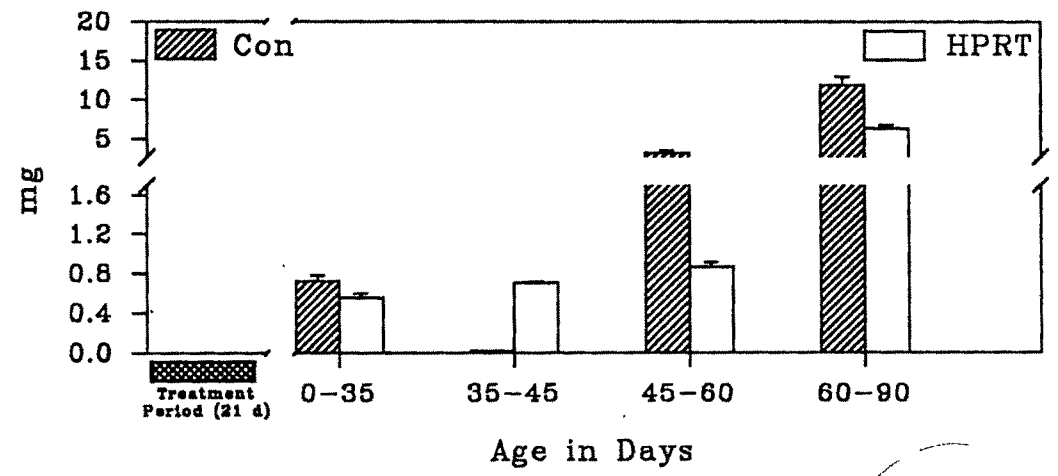

Fig. 8 Rate of growth of seminal vesicle in intact and HPRT rats

45 and 60 days in control animals and between 60 and 90 days in HPRT animals. The maximum relative weight of seminal vesicle at 35 days was in the HPRT rats. There was a decrement in relative weight in both the groups at 45 days and at 90 days, the relative weight was significantly less in HPRT animals compared to the controls.

Prostate Gland (Table 4.3 a, b; Fig. 9 a, b & 10)
The absolute weight of the prostate gland was significantly lowered in HPRT animals compared to the controls at 35 days. Though there was a decrease in the weight of the prostate gland at 45 days in control animals before, showing continuous increment, in HPRT animals, there was continuous increase. At 90 days, the final weight of prostate was significantly lowered in HPRT rats compared to the controls. 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 animals. In general, the relative weight of prostate paralleled the changes in absolute weight.

Thyroid Gland (Table 4.4; Fig. 11 a & b)
The HPRT animals recorded significantly less thyroid weight compared to the controls at 35 days. Though the HPRT 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 between 60 and 90 days in HPRT group of rats. Whereas the relative weight was similar in control and HPRT animals at 35 days, the same was high in HPRT animals compared to the controls at 90 days.

II. HISTOLOGICAL OBSERVATIONS

STRUCTURE OF TESTIS (Table 4.5; Plates I-III)

35 Day Old
Control: The tubules were small with an average diameter of 90.47 μm with mostly

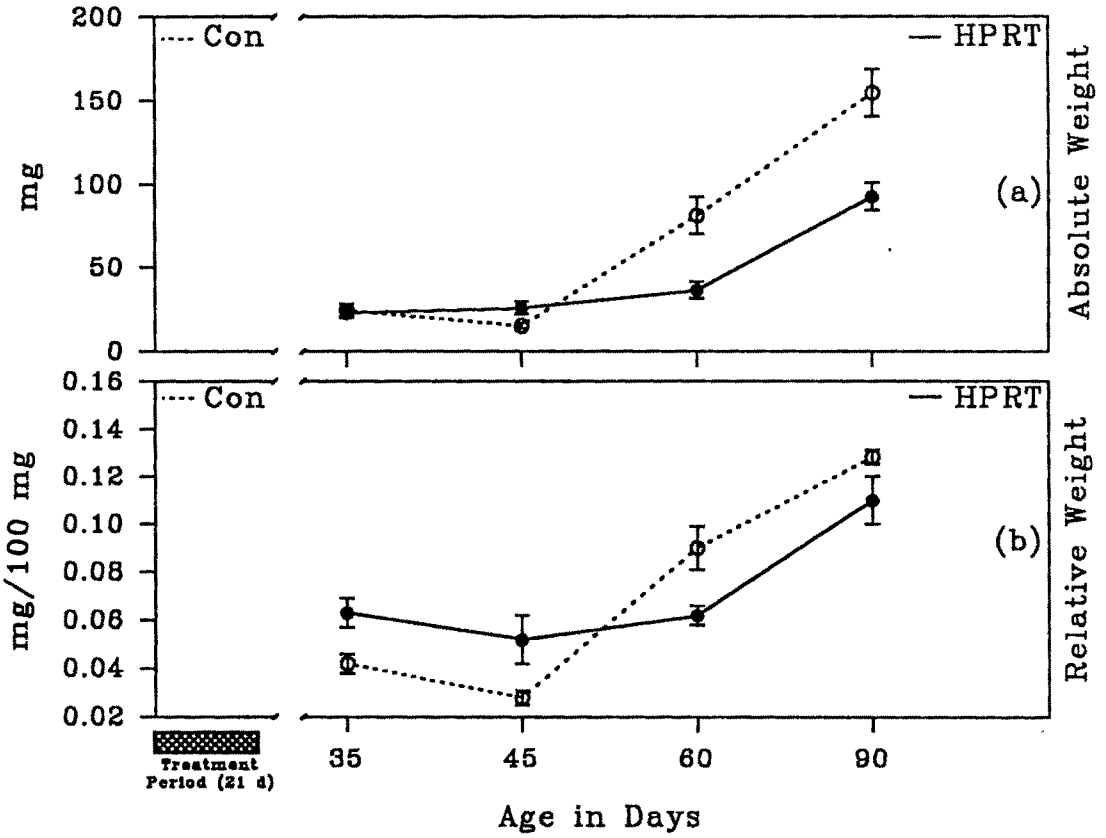

Fig. 9 (a&b) Chronological alterations in absolute and relative weights of prostate gland in intact and hyperthyroid (HPRT) rats

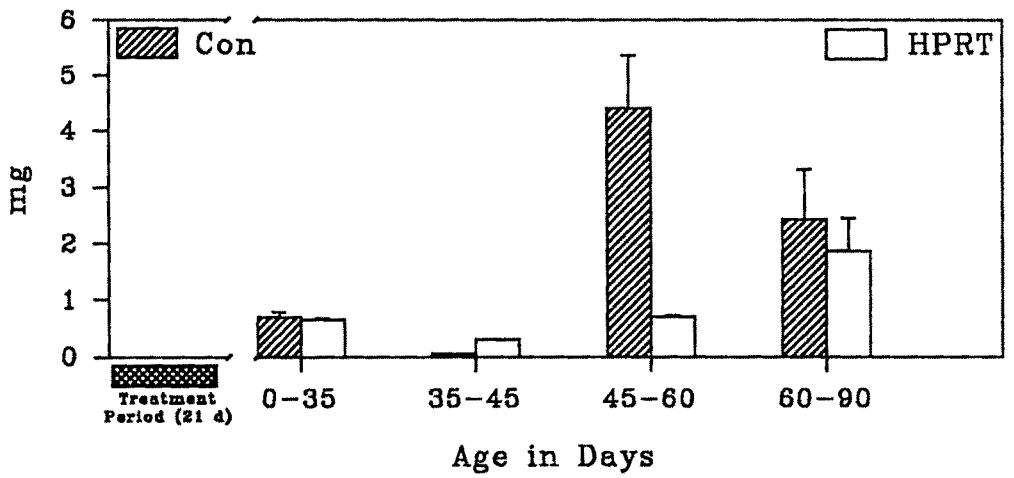

Fig. 10 Rate of growth of prostate gland in intact and HPRT rats

Table 4.4 Chronological alterations in Weight [Absolute (mg) and Relative (mg/100mg)] and Percentage Difference of Thyroid Gland in intact and hyperthyroid (HPRT) rats

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	0.01 ± 0.002	0.006 ± 0.001	0.007 ± 0.0006	0.007 ± 0.0005	- 41.33	+ 88.07	+ 22.36	+ 35.009
HPRT	4.68 ± 0.77^{d}	5.42 ± 0.35^{d}	5.56 ± 0.62^{d}	7.58 ± 0.79^{ns}	0.013 ± 0.002^{b}	0.011 ± 0.001^{d}	0.009 ± 0.001^{c}	0.009 ± 0.001^{c}	+ 15.81	+ 2.58	+ 36.33	+ 61.97

@ Values expressed as Mean ± SD of five experiments

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

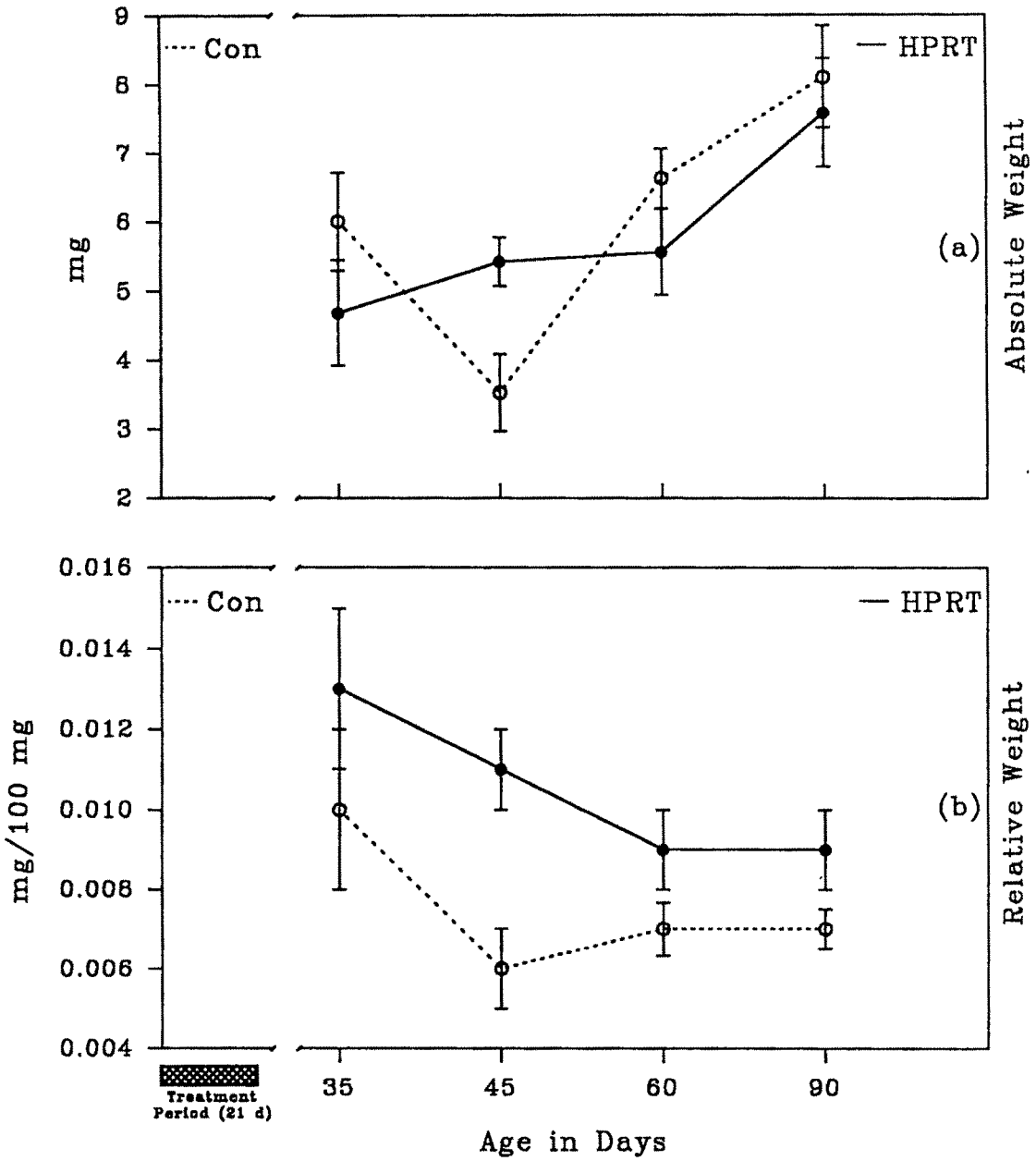

Fig. 11 (a&b) Chronological alterations in absolute and relative weights of thyroid gland in intact and hyperthyroid (HPRT) rats

Table 4.5 Chronological alterations in the Diameter (in μm) of Seminiferous Tubule and Epididymis (Caput and Cauda) in intact and hyperthyroid (HPRT) rats

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	110 47 ± 7.12^{c}	114.28 ± 9 26^{ns}	114.67 ± 12.36^{d}	168.86 ± 12 16^{a}	30.91 ± 2.67^{d}	31.57 ± 2.11^{b}	27.76 ± 2.52^{a}	32.48 ± 3.40^{d}	19.19 ± 1.76^{d}	20.47 ± 1.84^{d}	16.81 ± 1 61^{b}	17.00 ± 1.34^{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 1-5: Photomicrographs of sections of testis of 35 day old neonatally hyperthyroid (HPRT) rats.

Figures 1 and 2 (100 x); 3-5 (200 x): Sections of testis of 35 day old rat with well formed tubules and relatively more number of germ cells and more number of spermatocytes (Sc). Degenerating cells (arrow) are rare.

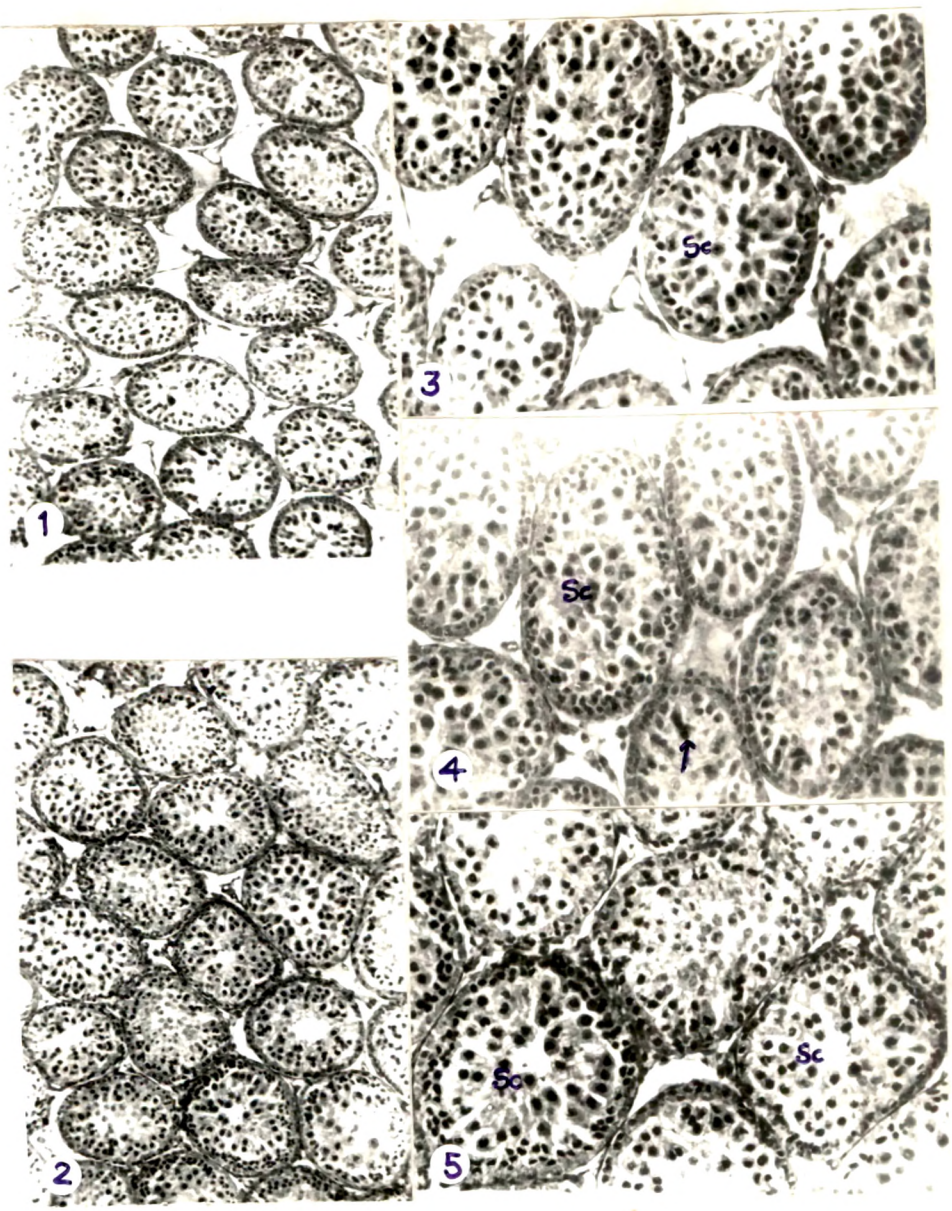
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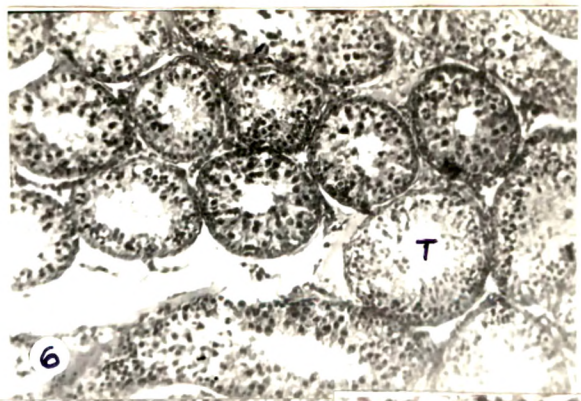
PLATE II

Figures 6-12: Photomicrographs of sections of testis of 45 and 60 day old neonatally hyperthyroid (HPRT) rats.

Figures 6 (100 x); 7 and 8 (200 x): Sections of testis of 45 day old rat with tubules (T) showing advanced stages of spermatogenesis and prominent interstitium (I). Note the overall decrease in germ cell population.

Figures 9 and 10 (100 x); 11 and 12 (200 x): Sections of testis of 60 day old rat showing tubules of the same size as at 45 days and progression of spermatogenesis up to round spermatids (RSt). Note the decrease in overall germ cell population.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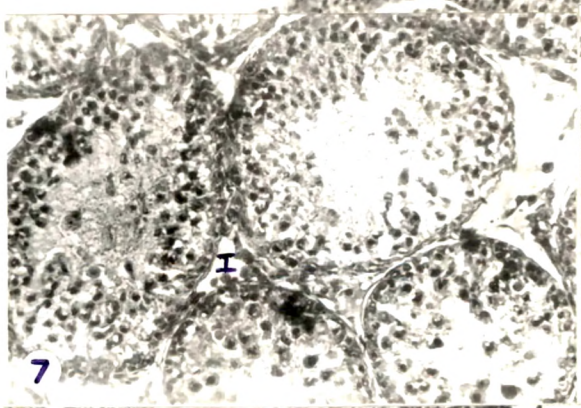
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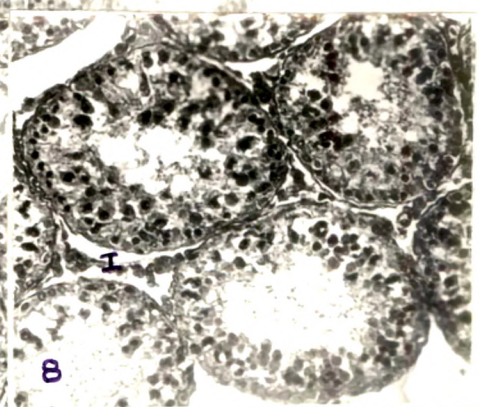
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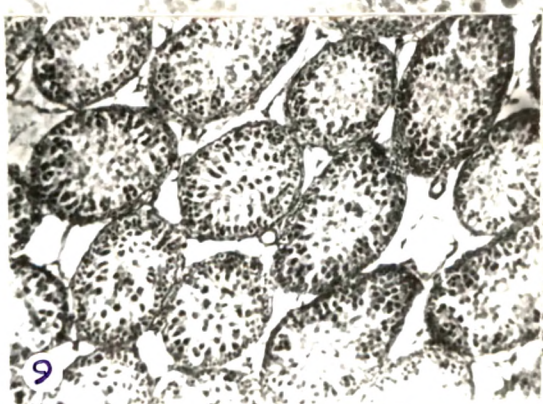
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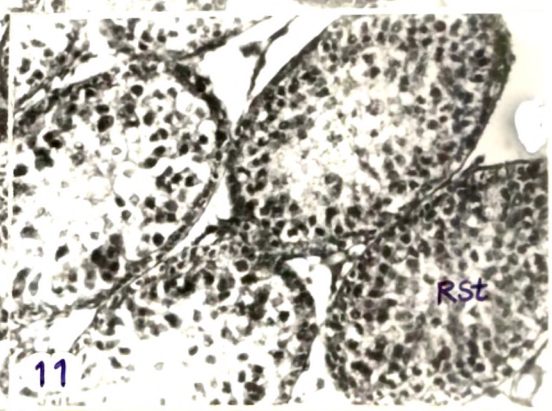
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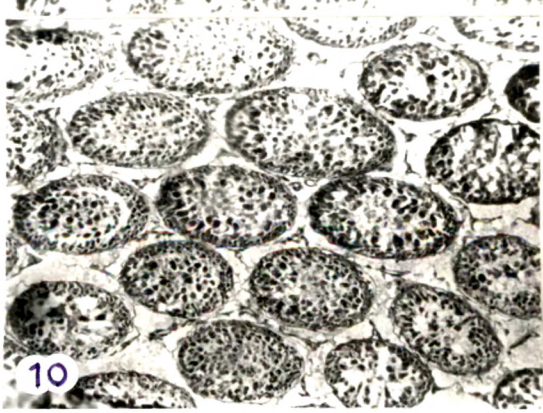
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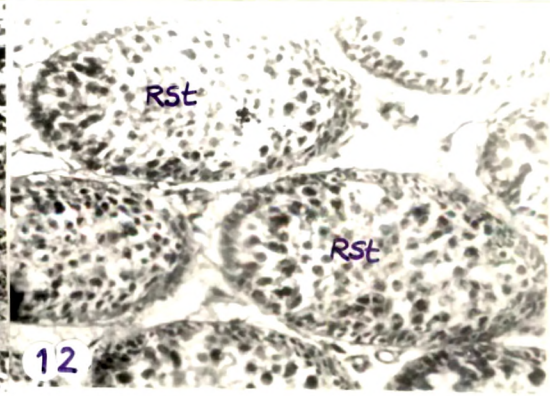
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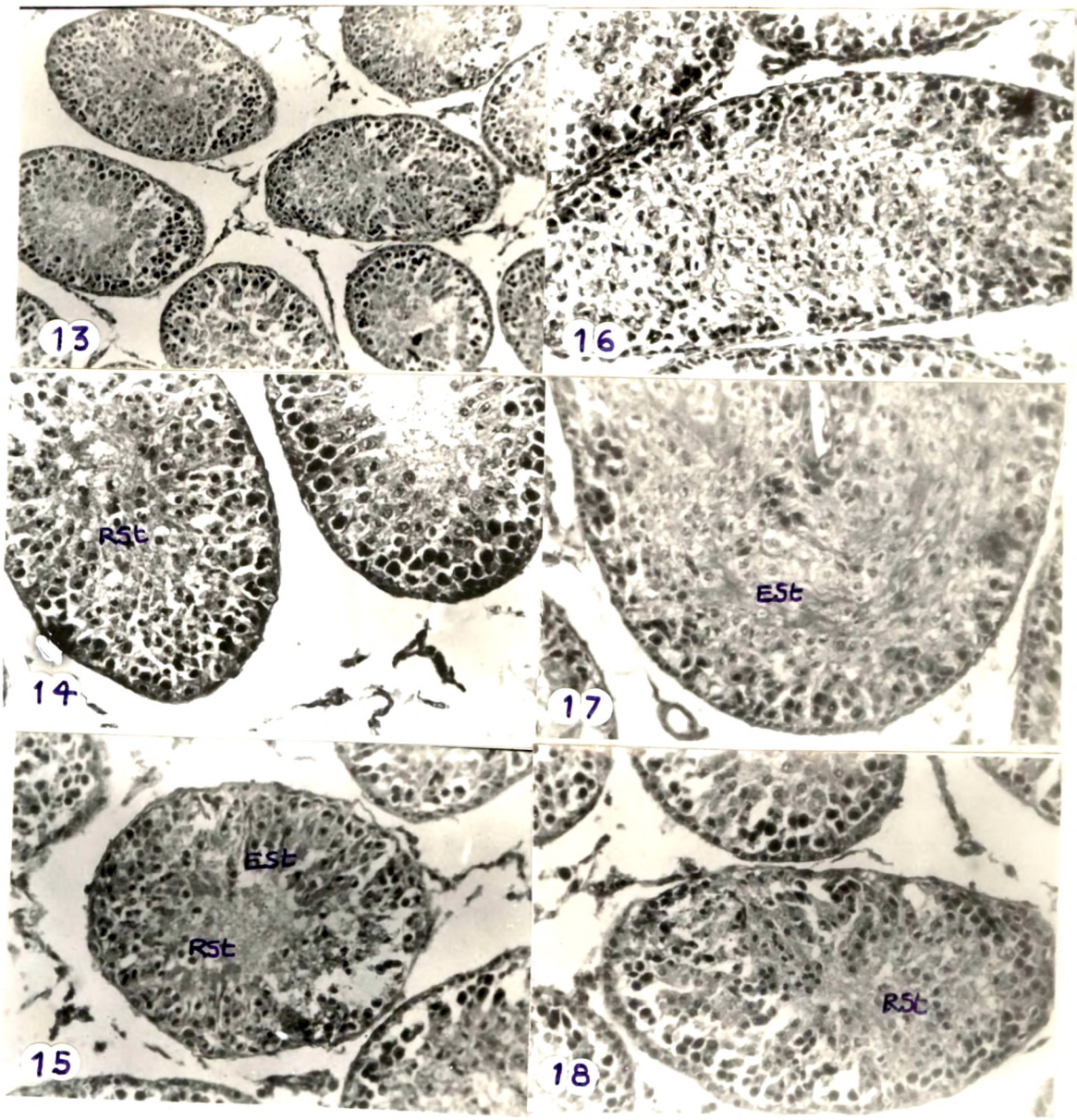
PLATE III

Figures 13-18: Photomicrographs of sections of testis of 90 day old neonatally hyperthyroid (HPRT) rats.

Figures 13 (100 x); 14-18 (200 x): Sections of testis of 90 day old rat showing relatively larger tubules with spermatogenesis well established showing round spermatids (RSt) and elongating spermatids (ESt). Note the overall less dense germ cell population and no spermatozoa.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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: The tubules were well formed with an average diameter of 110.47 μm. There were relatively more number of germ cells with many primary spermatocytes in the zygotene stage. Degeneration of germ cells was rare and overall picture more resembled that of Px. The interstitium also appeared to be better 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: The tubules appeared to be more or less of the same size as at 35 days and the average diameter was 114.28 μm. The germ cell population appeared to be less though spermatogenesis was found to be progressing towards advanced meiotic stages. The interstitial cells appeared to be hypertrophied.

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: The tubules were compactly packed and the average diameter remained the same as at 45 days (114.67 μm). Spermatogenesis had progressed up to round spermatids though the overall germ cell population was less.

90 Day Old

Control: The tubules were further enlarged with the 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.

HPRT: The tubules were larger and measured an average diameter of 168.86 μm. Spermatogenesis was well established and many of the tubules showed round and elongating spermatids though spermatozoa were not yet formed. The overall germ cell population appeared less dense. Spermatid differentiation appeared to be affected. The interstitium was moderately developed and appeared fibroblast like.

STRUCTURE OF EPIDIDYMIS (Table 4.5; Plate IV)

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: The tubules were tightly packed and the cells were well formed with an average cell height of 30.9 μm in the caput and 12.2 μm in the cauda. The lumen appeared empty.

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: The tubules were well formed and the epithelium appeared hypertrophied with an average cell height of 31.6 μm in the caput and 20.5 μm in the cauda. Germ cells could be seen in the lumen occasionally.

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.

PLATE IV

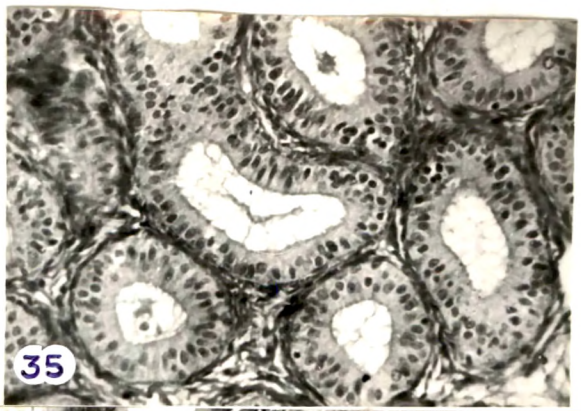
Figures 35-40: Photomicrographs of sections of epididymis of 35,45,60 and 90 day old neonatally hyperthyroid (HPRT) rats (200 x).

Figure 35: Section of epididymis of 35 day old rat showing tightly packed tubules with prominent epithelium and narrow lumen.

Figures 36 and 37: Sections of epididymis of 45 day old rat showing well formed tubules with slightly hypertrophied epithelium. Note the presence of degenerating germ cells (arrow) in the lumen.

Figure 38: Section of epididymis of 60 day old rat showing prominent tubules lined by large epithelial cells. Degenerating germ cells (arrow) could be seen in the lumen.

Figures 39 and 40: Sections of epididymis of 90 day old rat showing well formed tubules lined by large epithelial cells. The lumen appears narrow and contains some germ cells and secretory material (S).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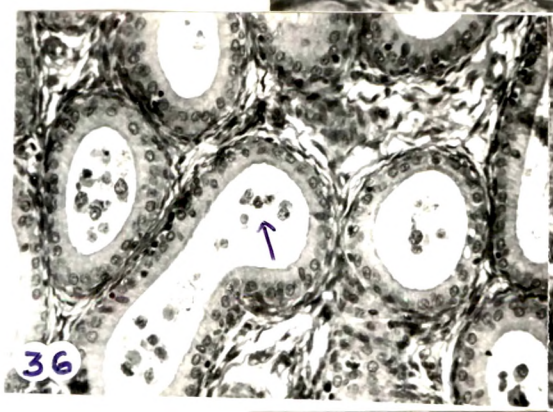
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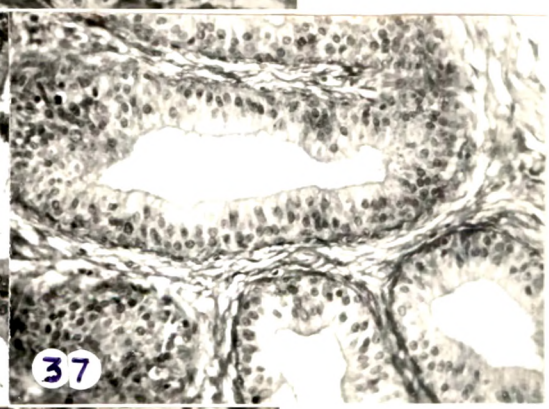
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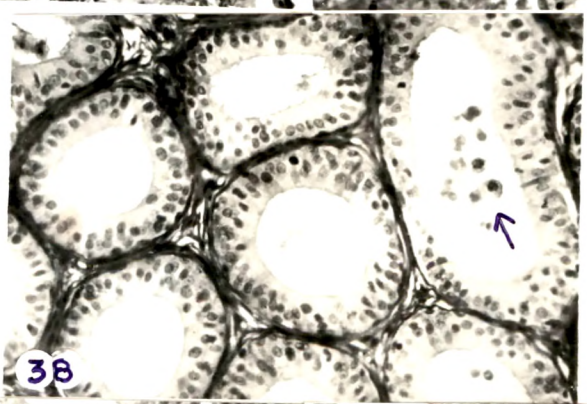
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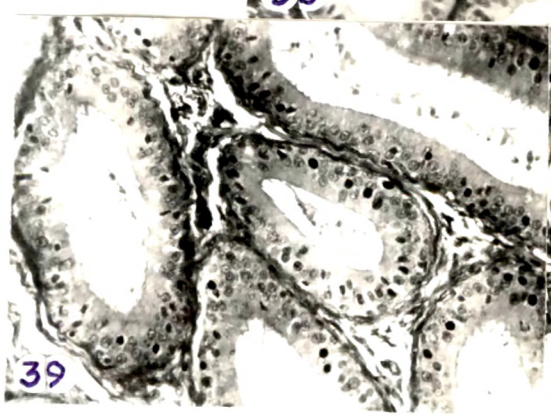
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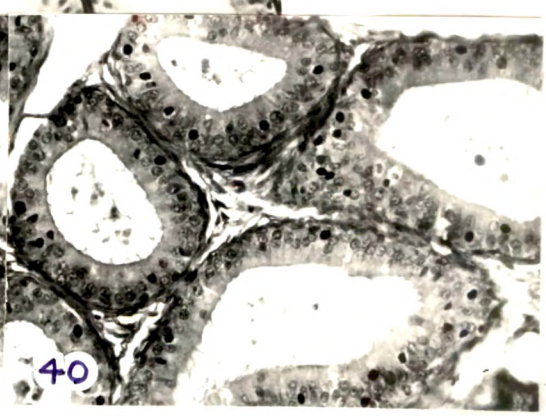
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HPRT: The tubules were prominent with large epithelial cells. The average cell height in the caput was 27.7 μm and in the cauda 16.8 μm. Degenerating germ cells could be seen occasionally in the lumen.

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: The tubules were well formed with large epithelial cells with the average cell height being 32.5 μm in the caput and 17 μm in the cauda. The lumen appeared narrow and contained some secretory material with germ cells.

STRUCTURE OF SEMINAL VESICLE (Plate V)

35 Day Old

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

HPRT: The secretory epithelium was well formed and convoluted with hypertrophied cells. The lumen contained secretory material.

45 Day Old

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

HPRT: The secretory epithelium was well developed with secretory material in the lumen. The cells appeared slightly smaller in size compared to the controls.

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: The epithelium was well developed but less convoluted and the cells were hypertrophied Reduced secretory material could be seen in the lumen.

PLATE V

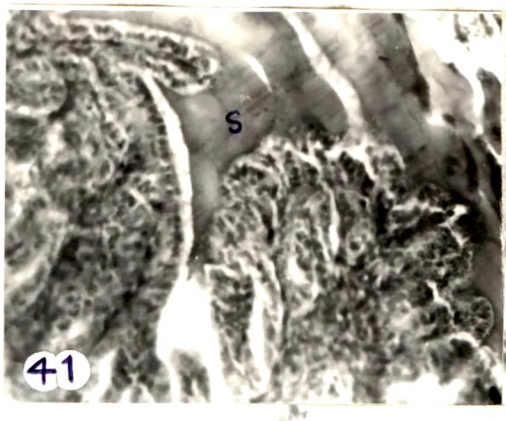
Figures 41-45: Photomicrographs of sections of seminal vesicle of 35,45,60 and 90 day old neonatally hyperthyroid (HPRT) rats (200 x).

Figures 41 and 42: Sections of seminal vesicle of 35 day old rat showing well formed convoluted secretory epithelium lined by hypertrophied cells. Secretory material (S) can be seen in the lumen.

Figure 43: Section of seminal vesicle of 45 day old rat showing convoluted secretory epithelium lined by smaller cells. Secretory material (S) could be seen in the lumen.

Figure 44: Section of seminal vesicle of 60 day old rat showing secretory epithelium lined by slightly hypertrophied cells. Holocrine (arrow) activity of the cells could be seen and there is very little secretory material (S).

Figure 45: Section of seminal vesicle of 90 day old rat showing well formed secretory epithelium with hypertrophied cells.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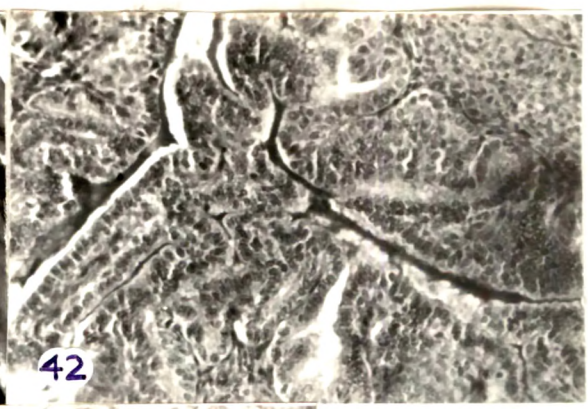
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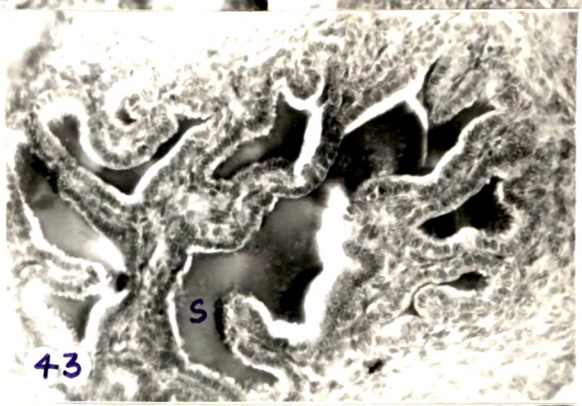
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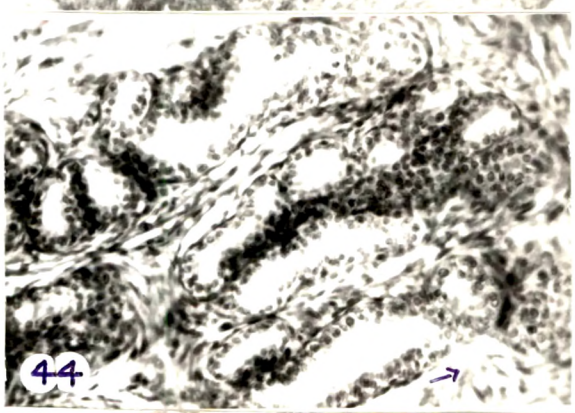
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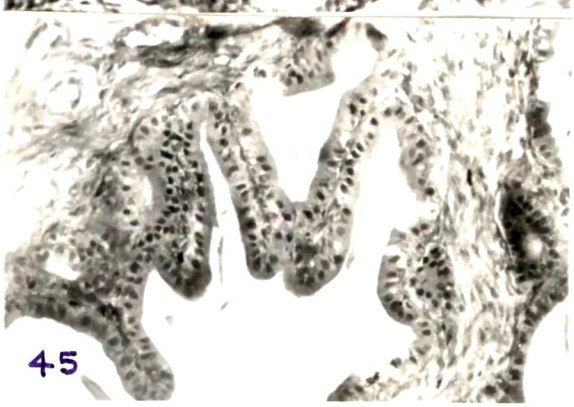
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90 Day Old

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: The epithelium was well formed with hypertrophied cells and secretory material could be seen in the lumen.

STRUCTURE OF PROSTATE (Plate VI)

35 Day Old

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

HPRT: The prostatic acini were lined by tall, hypertrophied, cuboidal and columnar pseudostratified epithelium.

45 Day Old

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

HPRT: The acini were well developed and lined by large cuboidal epithelial cells. Amorphous secretory material could be seen 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.

HPRT: The acini were lined by large cuboidal to columnar epithelial cells. The content of secretory material in the lumen was much less.

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.

PLATE VI

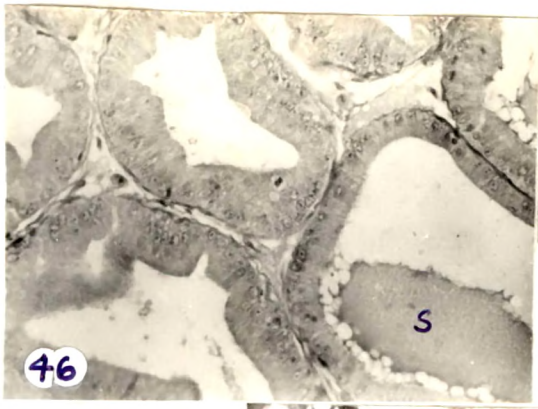
Figures 46-51: Photomicrographs of sections of prostate of 35,45,60 and 90 day old neonatally hyperthyroid (HPRT) rats (200 x).

Figures 46 and 47: Sections of prostate of 35 day old rat showing well formed prostatic acini lined by tall, hypertrophied, cuboidal and columnar epithelial cells. Secretory material (S) can be seen in the lumen.

Figure 48: Section of prostate of 45 day old rat showing well developed prostatic acini lined by large cuboidal cells.

Figure 49: Section of prostate of 60 day old rat showing compact prostatic acini lined by cuboidal epithelial cells. The secretory content is almost nil.

Figures 50 and 51: Sections of prostate of 90 day old rat showing large prostatic acini lined by cuboidal to columnar cells. Secretory material (S) could be seen in the lumen.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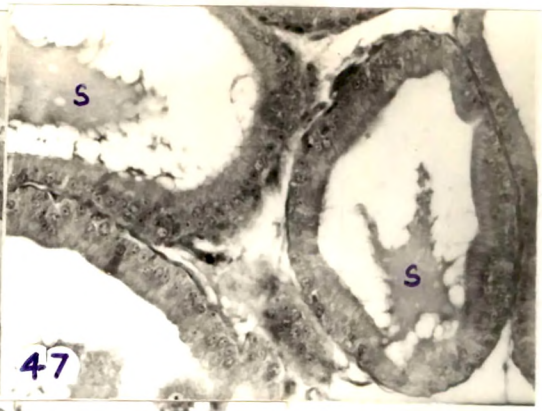
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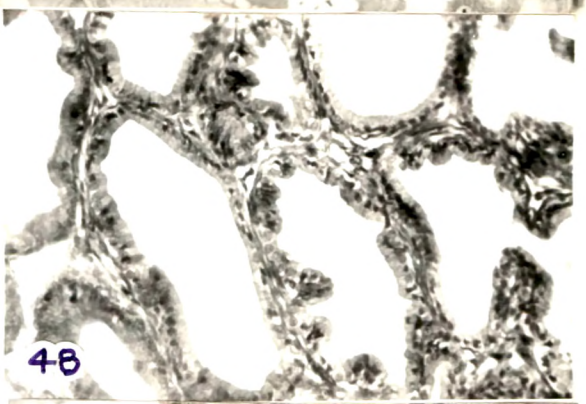
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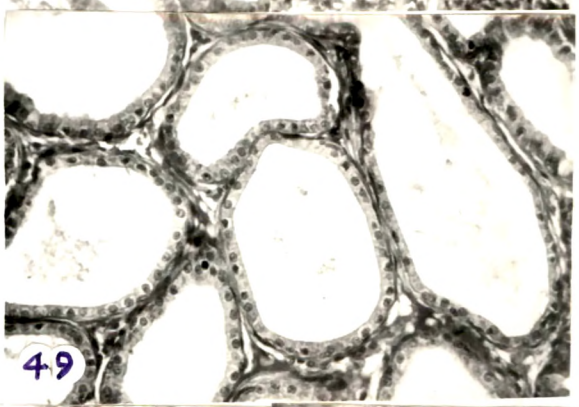
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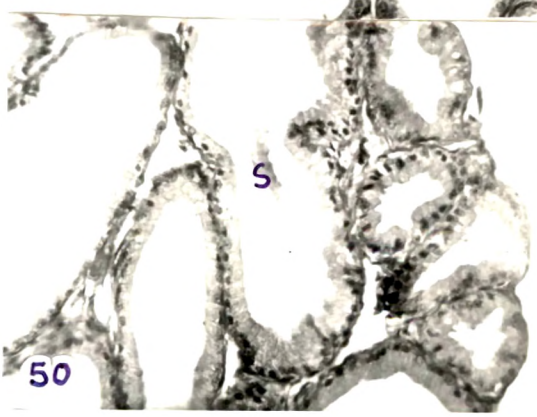
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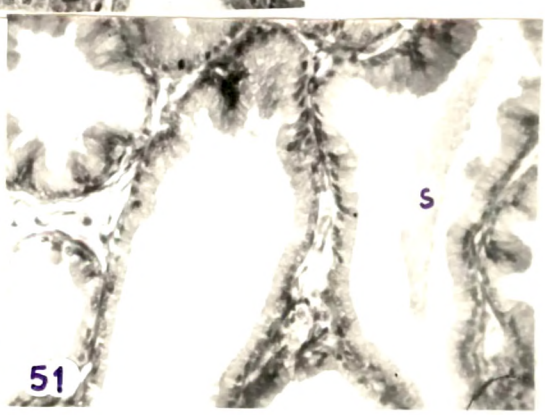
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HPRT: The acini were large and lined by cuboidal to columnar epithelium. Secretory material could be seen in the lumen.

STRUCTURE OF THYROID (Plate VII)

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

HPRT: The follicles were lined by cuboidal epithelium and contained colloid.

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

HPRT: The follicular epithelium was slightly hypertrophied and many of the follicles contained colloid.

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

HPRT: Follicles were lined by cuboidal epithelium and most of them were filled with colloid.

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

HPRT: The follicles were lined by large cuboidal epithelial cells. Some of the follicles were filled with colloid while others were empty. The overall colloid content was moderate.

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

PLATE VII

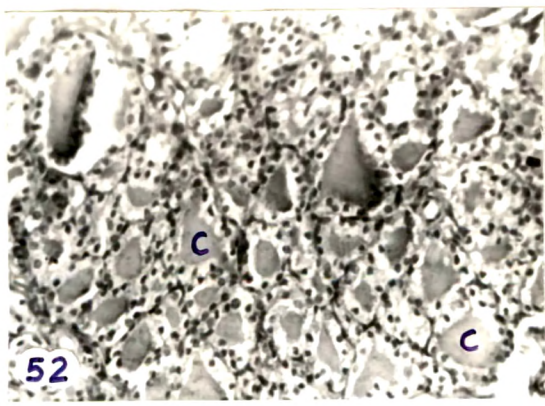
Figures 52-55: Photomicrographs of sections of thyroid of 35,45,60 and 90 day old neonatally hyperthyroid (HPRT) rats (200 x).

Figure 52: Section of thyroid of 35 day old rat showing follicles lined by cuboidal epithelium and presence of colloid (C).

Figure 53: Section of thyroid of 45 day old rat showing follicles lined by slightly hypertrophied epithelium and containing colloid (C).

Figure 54: Section of thyroid of 60 day old rat showing follicles lined by cuboidal epithelium. Almost all the follicles contain colloid (C).

Figure 55: Section of thyroid of 90 day old rat showing follicles lined by large active cuboidal epithelial cells and containing differential colloid (C) content.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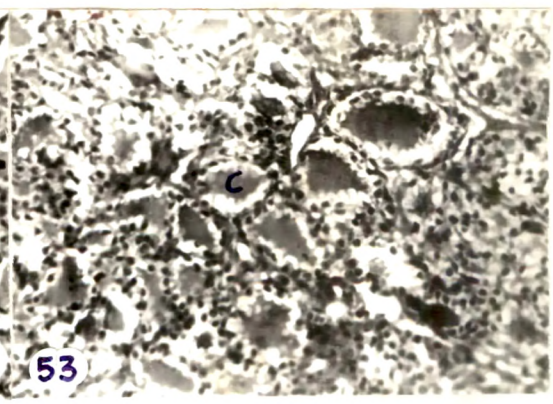
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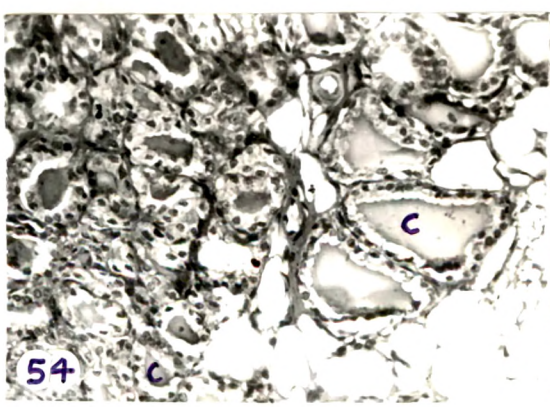
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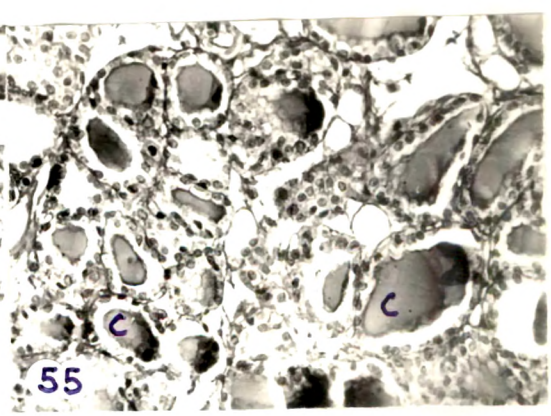
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PLATE VIII

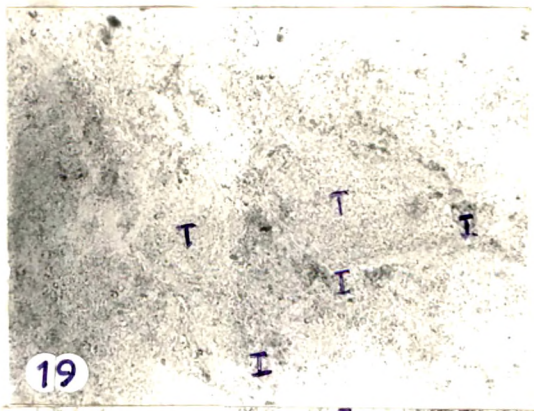
Figures 19-26: Photomicrographs of sections of testis of 35,45,60 and 90 day old neonatally hyperthyroid (HPRT) rats showing histochemical localisation of 3α and 17ß HSDH respectively (65 x).

Figures 19 and 23: Sections of testis of 35 day old rat showing 3α HSDH (Fig.19) and 17ß HSDH (Fig.23) respectively. Note the feeble activity in the interstitium (I) and no activity in the tubules for 3α HSDH. There is discernible activity in the tubules (T) and mild activity in the interstitium (I) for 17ß HSDH.

Figures 20 and 24: Sections of testis of 45 day old rat showing 3α HSDH (Fig.20) and 17ß HSDH (Fig.24) activity respectively. Note the intense activity of both the enzymes in the tubules (T) and almost very weak activity in the interstitium (I).

Figures 21 and 25: Sections of testis of 60 day old rat showing 3α HSDH (Fig.21) and 17ß HSDH (Fig.25) respectively. Note the intense activity of 3α HSDH in the tubules (T) containing advanced stages of spermatogenesis. There is noticeable activity of 17ß HSDH in the interstitium (I).

Figures 22 and 26: Sections of testis of 90 day old rat showing 3α HSDH (Fig.22) and 17ß HSDH (Fig.26). Note the intense 3α HSDH activity in the tubules (T) containing advanced stages of spermatogenesis and the moderate response of 17ß HSDH in the tubules (T). The activity of both the enzymes in the interstitium was weak.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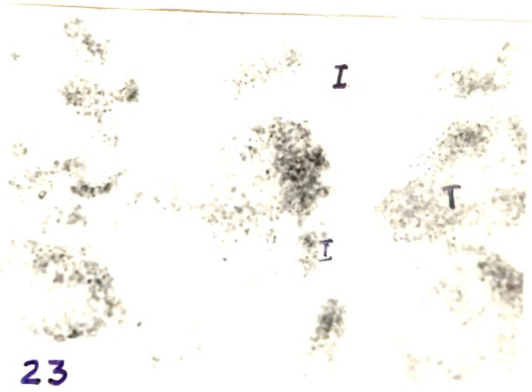
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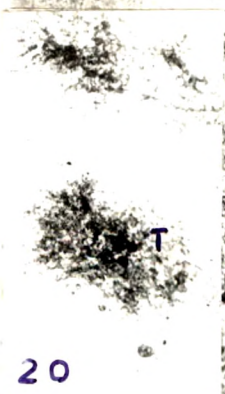
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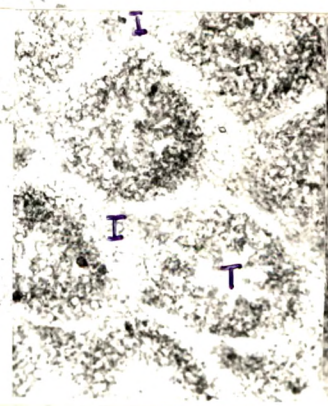
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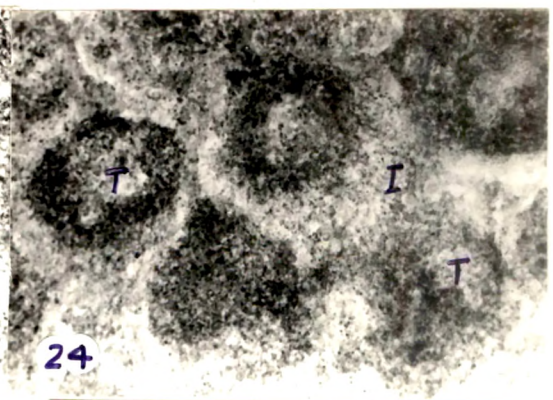
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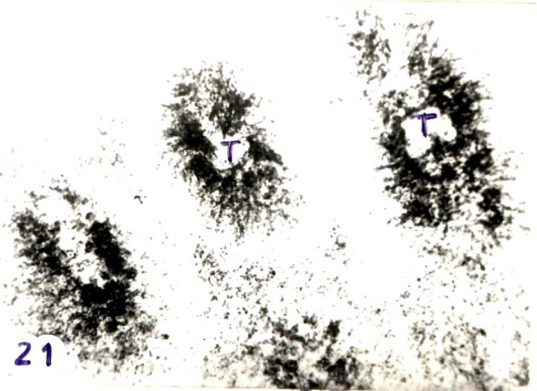
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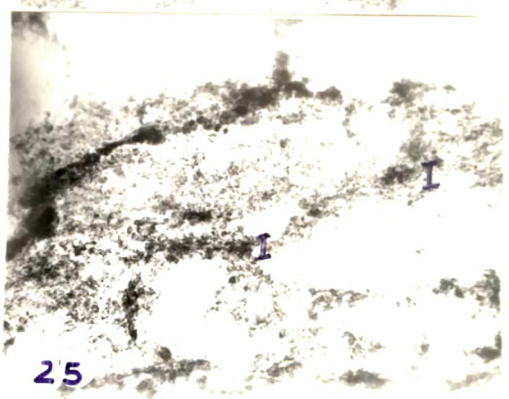
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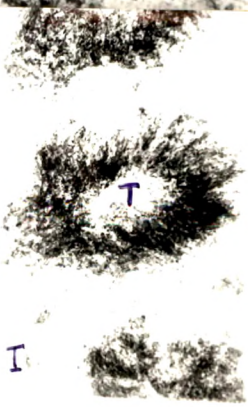
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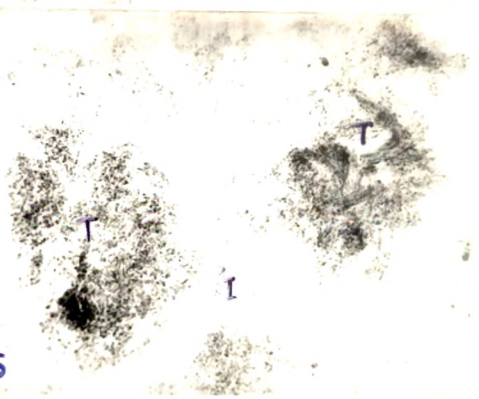
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PLATE IX

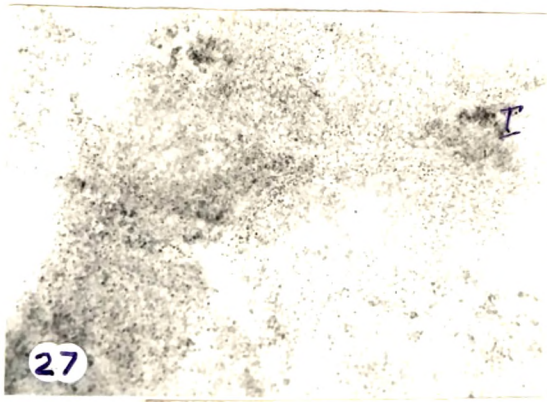
Figures 27-34: Photomicrographs of sections of testis of 35,45,60 and 90 day old neonatally hyperthyroid (HPRT) rats showing 3ß HSDH localisation with P and DHEA as substrates (65 x).

Figures 27 and 31: Sections of testis of 35 day old rat showing noticeable activity in the interstitium (I), more prominent with DHEA as substrate (Fig.31).

Figures 28 and 32: Sections of testis of 45 day old rat showing activity of both the substrates in the tubules (T).

Figures 29 and 33: Sections of testis of 60 day old rat showing intense activity of 3ß HSDH in both the tubules (T) and interstitium (I), more prominent with DHEA (Fig.33).

Figures 30 and 34: Sections of testis of 90 day old rat showing moderate to intense activity in the tubules (T) having advanced stages and a weak activity in the interstitium (I).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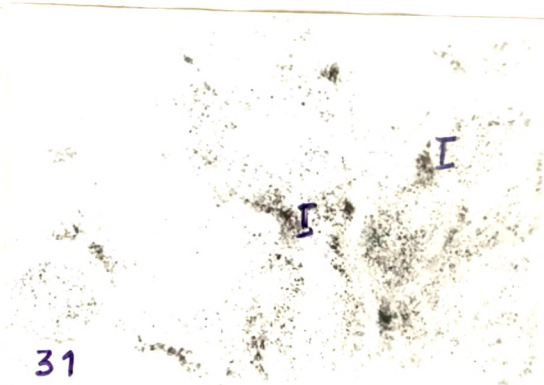
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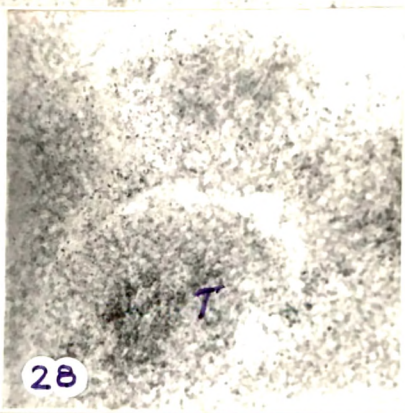
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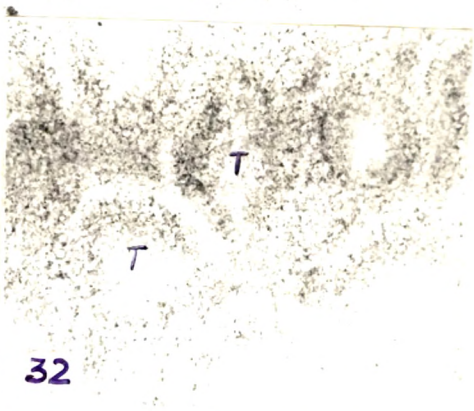
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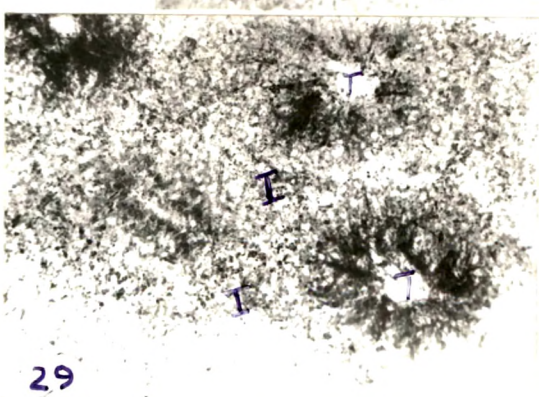
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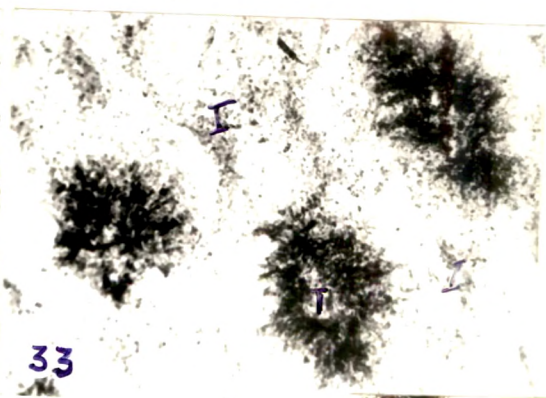
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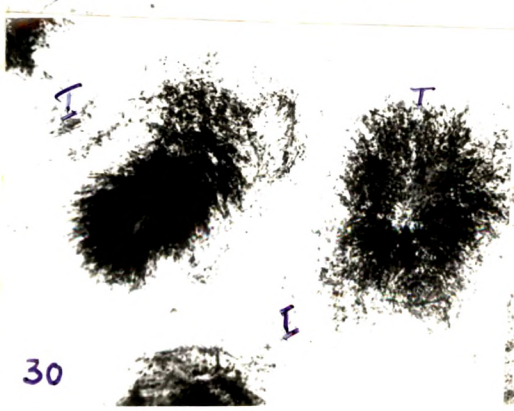
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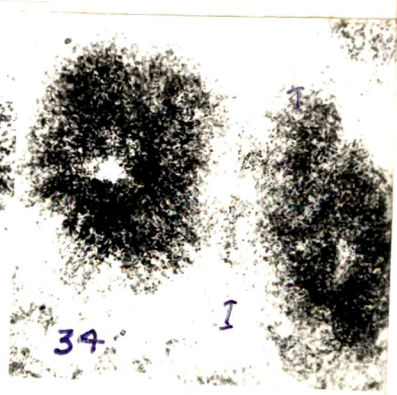
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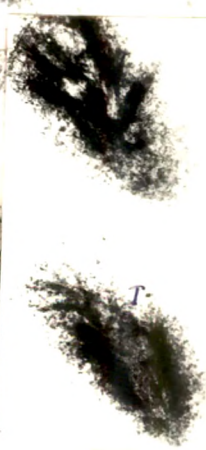

discernible with DHEA as the substrate but not with P. The 17ß-HSDH activity was appreciable in the tubules while no activity was visible in the Leydig cells in the control rats. The 3α-HSDH activity was weakly localised in the tubules but not in the Leydig cells of control rats. In the HPRT rats, the 3ß-HSDH activity was prominent in the interstitium and significantly greater with DHEA as the substrate than with P. In contrast, the 17ß-HSDH activity was weak in the interstitium but clearly discernible in the tubules. There was no activity of 3α-HSDH in the tubules and very little in the interstitium.

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 the substrate which was not evident with P. In the control animals, the Leydig cells were weakly enzyme active while the tubules showed significant 17ß-HSDH 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 animals. In the HPRT rats, there was noticeable activity of 3ß-HSDH in the tubules and a weak activity in the interstitium, more prominent with DHEA as the substrate. 17ß-HSDH showed strong localization in the tubules while it was feeble in the interstitium. The tubules showed varying but intense response towards 3α-HSDH, but the interstitium was negative.

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. The tubules of control rats 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 rats, strong localization of 3ß-HSDH in both the tubules and the interstitium was the feature, clearly more with DHEA. There was mild but noticeable activity of 17ß-HSDH in the interstitium while intense activity of the enzyme was discernible in tubules containing advanced stages of germ cells. 3α-HSDH also depicted a similar localization in the tubules and the interstitium responded weakly to this enzyme.

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 animals, 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 rats, all the three enzymes showed strong activity only in those tubules containing advanced stages of germ cells and were clearly localised in the central part towards the lumen. Similarly all the three enzymes showed moderate activity in the interstitium.

IV. SERUM HORMONE PROFILE (Table 4.6; 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 HPRT rats had significantly

Table 4.6 Chronological alterations in Serum Triiodothyronine, Thyroxine and Testosterone levels in intact and hyperthyroid (HPRT) rats

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	1.95 ± 0.27^{b}	3.60 ± 0.41^{c}	3.60 ± 0.38^{c}	2.50 ± 0.19^{ns}	36.48 ± 3.10^{d}	46.02 ± 2.50^{d}	45.30 ± 3.30^{d}	41.49 ± 3.00^{d}	0.41 ± 0.11^{ns}	0.90 ± 0.13^{d}	1.37 ± 0.23^{d}	1.36 ± 0.28^{ns}

@ Values expressed as Mean ± SD of five experiments

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

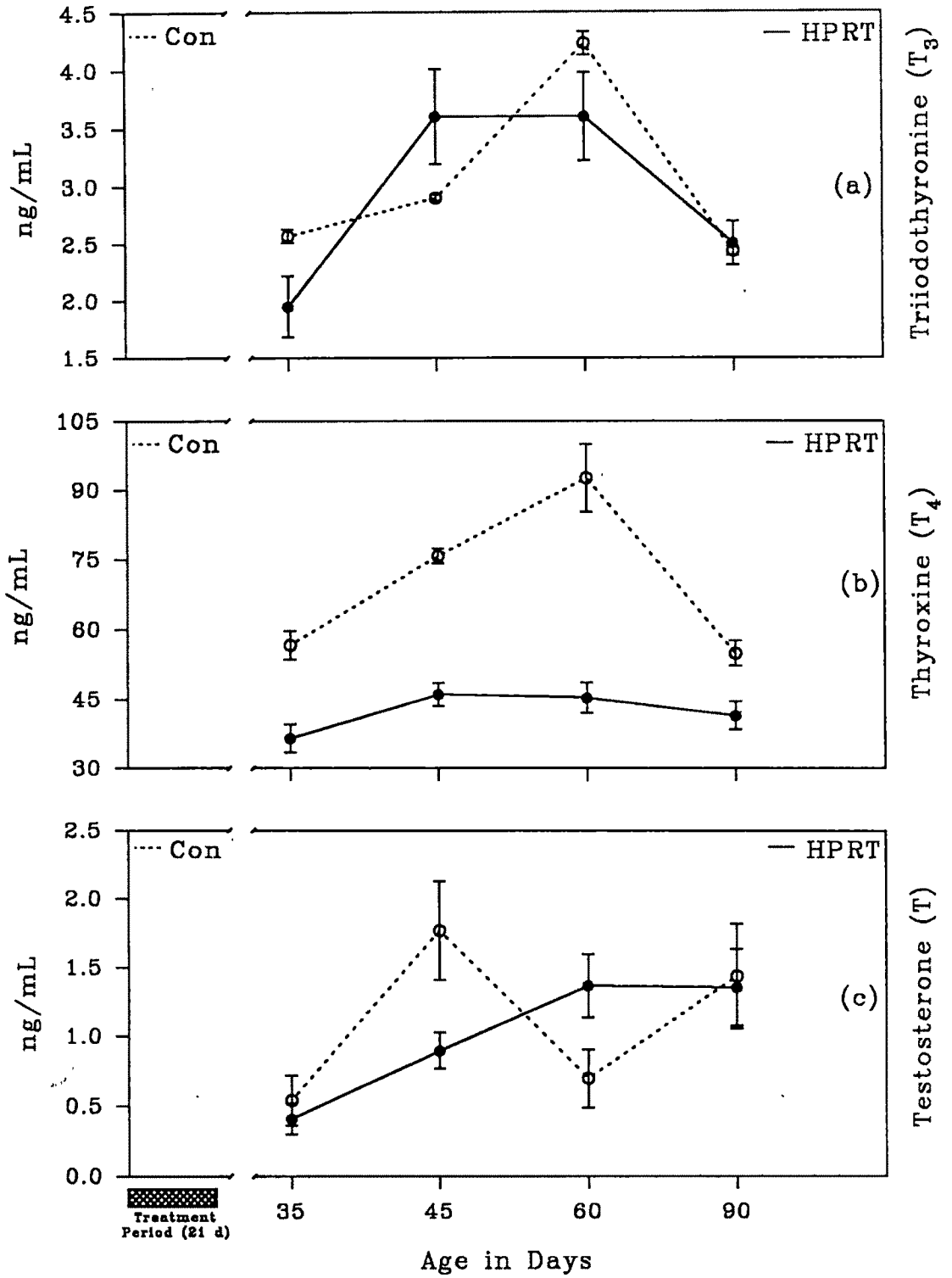

Fig. 12 (a, b & c) Chronological alterations in serum T_3, T_4 and T levels in intact and hyperthyroid (HPRT) rats

lowered T_3 and T_4 levels at 35 days. However, whereas the serum T_3 level remained in the control range from 45 days onwards, the serum T_4 level increased only marginally and remained at the same significantly low level through out 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 serum T level was also slightly lower in the HPRT animals at 35 days whereafter it increased significantly to attain a peak level by 60 days. The T level remained constant thereafter. Compared to the controls the T level was lower than the controls at 35 and 45 days.

DISCUSSION

Recent studies on Long-Evans and Sprague-Dawley strains of rats had demonstrated an hypertrophied growth, resulting in larger adult testis size in rats rendered transiently hypothyroid neonatally (Cooke and Meisami, 1991; Cooke *et al.*, 1992; 1993; Meisami *et al.*, 1992; Kirby *et al.*, 1992; Hess *et al.*, 1993). This effect was related to a prolonged phase of Sertoli cell proliferation resulting in increased number of Sertoli cells and the cohort germ cells (Van Haaster *et al.*, 1992; Cooke *et al.*, 1994; de Franca *et al.*, 1995). However, our previous study on neonatal hypothyroidism in the Charles foster strain not only failed to produce these effects but resulted in much reduced testis size (chapter 1). Nevertheless, neonatal Px resulted in increased adult testis size, evidently by increasing the number of Sertoli and germ cells (chapter 2), while hypothyroidism in pinealectomised neonates led to no change in the adult testis size but increased the overall germ cell population (chapter 3). The present study undertaken as a corollary to the hypothyroid studies, again resulted in ultimate reduced adult testes size and lesser number of germ cells. There was also a delay in the establishment of the spermatogenic process as revealed by the appearance of differentiating spermatids only by 90 days as against 45 days in the controls.

The hyperthyroid rats showed retarded body and testes weights with significantly reduced growth rates and, at 90 days, the body and testes weights were only 70 and 67% respectively, of the controls. Microscopic observations of sections of testis have shown increased germ cell number and diameter of seminiferous tubules at 35 days. By 45 days, the tubular diameter was identical to that of controls and there was significantly lesser number of germ cells with more degenerating germ cells. At 60 days spermatogenesis appeared to be reestablished and by 90 days had progressed only up to the stage of elongating spermatids. In contrast, spermatids were formed by 45 days and spermatozoa by 60 days in control animals. The diameter of the tubules was significantly less at 60 and 90 days. Explanation for these observations should await a consideration of the effects of neonatal hyperthyroidism on thyroid axis as well as the perturbations on other hormones of consequence. The hypothalamic-pituitary-thyroid axis develops and matures in the neonatal period (Dussault and Labrie,1975) and the first few postnatal days are considered critical for the future integrity of this axis (Eayrs and Holmes, 1964). In this context, neonatal hypo-or hyper-thyroidism could affect the development of this axis and as such a study on induced neonatal hyperthyroidism has shown reduced serum TSH. thyroxine and triiodothyronine levels after the withdrawal of thyroxine treatment extending to the adult stage. Apparently, neonatal hyperthyroidism renders the animals hypothyroidic in the adult stage and has been suggested to be possibly due to a permanent resetting of the regulatory set-point for pituitary TSH secretion and to increased sensitivity to the feedback inhibitory effects of thyroid hormones (Dussault *et al.*, 1982). This effect of neonatal hyperthyroidism is well corroborated by the present results of decreased serum thyroxine and triiodothyronine levels from 35 to 90 days. The above workers used a dosage of 0.4 μg/gm of thyroxine for 12 days from the day of birth while in the present study a dosage of 0.02 μg/gm was used from day 1 to 21. The induction of a hypothyroidic state subsequent to the cessation of thyroxine treatment and extending to adulthood, testifies to the fact that, the neonatal hypothalamic-pituitary-thyroid axis is very sensitive towards thyroid hormone as, a 20 times lower dose employed in the present

study was still effective. Neonatal hyperthyroidism may also decrease the secretion of GH by affecting mechanisms regulating GH secretion (see, Giustina and Wehrenberg,1995). In this context, the permanently impaired body growth and the decreased body weight in the HPRT rats could be a consequence of reduced GH level and/or even reduced somatomedins as hypothesised by Dussault *et al.* (1982).

In terms of serum gonadotropin profile, reduced FSH and LH titres during the period of induced hyperthyroidism and their levels apparently returning to control levels only after 35 days have been purported as such reductions in gonadotropin levels due to thyroxine administration has been shown in juvenile, prepubertal and pubertal rats (Aruldhas *et al.*,). In our previous study on neonatal Px, increase in tubular diameter and germ cell number in the early phases was attributed to increased FSH levels (chapter 2). The presently observed increased tubular diameter at 35 days in this context appears a bit intriguing. It is likely that in a background of mild hyperthyroidism, FSH action may be potentiated and hence even subnormal FSH level could exert a positive influence on germ cell proliferation and tubule diameter. However, the ability of thyroid hormones to exert a protective influence against germ cell degeneration is apparent from the histological observations of very few degenerating germ cells at 35 days. An alternative possibility for the increased germ cell number and tubular diameter at 35 days could be the favourable influence of increased thyroid hormone status. A protective action of thyroid hormones on germ cell degeneration was also shown by Jannini *et al* .(1993) in their *in vivo* and *in vitro* studies on short term thyroxine administration to rat neonates

It is well established that the survival and sustenance of the late meiotic and post-meiotic germ cells are dependant on the presence of fully differentiated and mature Sertoli cells (chapters 2 and 3). The presently observed reduced population of germ cells at 45 days is due to increased germ cell degeneration between 35 and 45 days, in this context, suggests incomplete Sertoli cell differentiation. This is understandable when viewed with our previous

observation of a critical dependence by the Charles foster strain on FSH for Sertoli cell proliferation and differentiation, though an optimum level of thyroid hormone is necessary for the FSH action (chapters 1-3). Inferably, the decreased FSH level despite the hyperthyroidic state retards Sertoli cell proliferation and differentiation. Paradoxically when the FSH level was returned to normalcy (presumably from 35 day onwards) the thyroid hormone is low. Clearly, Sertoli cell differentiation and maturation occurs only by 45 days with the increase in serum thyroid hormone level to a permissive, minimal optimum level. The proliferation of Sertoli cells is also apparently delayed and remains restricted to a narrow window between the increase in FSH level and the increase in thyroid hormones. This once again strengthens our previous conclusion that in the Charles foster strain, FSH alone is the potent stimulator of Sertoli cell proliferation while, thyroid hormone is a necessary permissive factor for FSH induced Sertoli cell differentiation which is in contrast to the other strains of rats where, T_3 was shown to be the principal hormone (Cooke *et al.*, 1994; Palmero *et al.*, 1989;1995).

The histochemical observations also reflect the serum gonadotropin and T profiles. In the testis of control animals, 3ß-HSDH activity in the interstitium and 17ß-HSDH activity in the tubules are prominently localised at 35 days. But in HPRT animals the activities of these enzyme were week at 35 days and became prominent only by 45 days. Generation of intra-tubular T by 17ß catalysis using the androstenedione provided by the Leydig cells and the 3α metabolites of T by the action of 3α-HSDH were inferred to be important in the pre-pubertal stage (chapters 1-3). Apparently, with the late increase in the gonadotropin levels, the induction of 3ß and 17ß-HSDH activities occur by 45 days and the histochemical profile of the 45 day HPRT rats is comparable to that of 35 day old control animals. These histochemical observations also provide supportive evidence for the above contentions.

With the attainment of normal levels of serum gonadotropins, the spermatogenic functions get reestablished after 45 days, as could be seen from the histological features at 60 days. The

delayed Sertoli cell maturation and the consequent sluggishness in the establishment of spermatogenesis is well evidenced by the occurrence of only elongating spermatids at 90 days. Compared to our previous study on, neonatal hypothyroidism induced delay in Sertoli cell maturation and spermatogenesis, the presently observed delay on these aspects due to neonatal hyperthyroidism appears to be more protracted (chapter 1). This is apparently due to the slower return of gonadotropin levels to control levels in HPRT as compared to HPOT.

There is obvious growth retardation of the accessory glands which was very pronounced at 60 days. Though there was an increased growth rate between 60 and 90 days, the weights of all the organs were still less than the controls. Compared to the controls, the difference in weight was 22% for the epididymis, 40% for the prostate and 46% for the seminal vesicle. The increased weight of all the three organs at 45 days is essentially due to the decreased weight in the control, a strain characteristic as shown earlier (chapter 1). Though a role for thyroid hormone on the growth of accessory glands in the juvenile and pre-pubertal period was envisaged (chapter 1), at later study discounted this possibility as neonatal Px increased the weights of sex accessory organs despite the reduced thyroid hormone levels (chapter 2). This was explained to be due to the growth retarding influence of melatonin and its potentiation in the absence of thyroid hormone (chapter 3). Apparently thyroid hormone regulates sex accessory organ growth indirectly by resisting the growth inhibitory influence of melatonin. In the earlier study a parallelism between the time scale of increase in thyroid hormone and the establishment of the pineal melatonin rhythm (10-12 days) was considered to have some relationship between these two hormones in the infantile period (chapter 2). In a recent study Catala *et al.* (1988) has shown that T_3 can induce release of melatonin from 14 day old pineal glands. They had suggested that thyroid hormone might have a definite but different influence in the infantile period as, in the adult condition thyroid hormone is known to decrease melatonin levels (Catala *et al.*, 1987). Just as the previously discussed action of increased T_4 level in the neonatal period which leads to a resetting of the hypothalamic-pituitary-thyroid axis, it is likely that neonatal

hyperthyroidism might also lead to a resetting of the establishment of the pineal-melatonin rhythm. As a consequence there could be an advancement of the establishment of the pineal-melatonin rhythm and also an increased pineal melatonin output. Viewed in this perspective the melatonin level in the HPRT rats could be expectedly higher which should have resulted in reduced accessory organ growth due to the retardatory influence of melatonin. However, due to the concurrent hyperthyroidism due to T_4 administration, the growth retardatory influence of melatonin is minimised. This is evident by the presently recorded weights of the accessory organs at 35 days as compared to hypothyroid rats where melatonin action was more potentiated and organ weights were drastically reduced (chapter 1). The histological features of these organs were clearly evident in the HPRT animals at 35 and 45 days. This could represent either delayed growth stimulation or a counter response to the growth retarding influence of melatonin, probably mediated by PRL as, PRL receptors have been located on the accessory organs even in the immature rats and PRL is known to be growth regulator of these organs (Kharroubi and Slaunwhite, 1984; Costello and Franklin, 1994; Reiter *et al.*, 1996). The PRL level incidently in the HPRT animals is elevated.

A scrutiny of the growth rates of the accessory organs in the control animals at different time periods reveals maximum growth rate between 45 and 60 days, though in the case of seminal vesicle it extends up to 90 days. In the HPRT rats, the growth rates were significantly low till 60 days and then registered peak rates between 60 and 90 days. The maximal growth rates of the accessory glands between 45 and 60 days in the control animals was accredited to a synergistic action of PRL and T (chapter 1). In the HPRT animals, though PRL levels were inferably elevated, the elevation of T levels was slow and attained the maximum level only by 60 days. This could explain the delayed growth response of the accessory glands which is again in keeping with the delay in testicular maturation observed earlier.

Overall, the present study reveals a delayed testicular maturation in HPRT animals due to the probably reduced FSH and LH levels. This also results in delayed sex accessory organ growth. It also appears that neonatal hyperthyroidism can advance the pineal-melatonin rhythm and also increase melatonin secretion.