CHAPTER 2

NEONATAL PINEALECTOMY RESULTS IN INCREASED ADULT TESTES AND ACCESSORY ORGAN SIZE IN THE CHARLES FOSTER STRAIN OF RAT

From the time pineal was recognised as an endocrine gland (Tinely and Waren, 1991), most of the earlier studies were in relation to the pineal and sex gland (Thieblot, 1960; Roth, 1965; Motta *et al.*, 1967; Reiter, 1981). Pinealectomy (Px) has been shown to increase reproductive activity in mammals (Reiter, 1973; 1980; Reiter *et al.*, 1985). Nevertheless, pinealectomy of the adult rats seems to have little or no stimulatory influence on the gonads (Motta *et al.*, 1967; Pitis and Maya, 1969; Reiter, 1973; 1980; Reiter *et al.*, 1985). However, the antigonadal properties of the pineal is best revealed in pre-pubertal rats as pinealectomy at this period tended to hasten puberty and increase the weight of gonads and accessory sex glands (Motta *et al.*, 1967; Pitis and Maya, 1969; Kinson, 1976). Further, administration of melatonin in the early pre-pubertal period has been shown to delay sexual maturation in the rat (Lang *et al.*, 1983; 1984; Rivest *et al.*, 1985). The period at which melatonin exerts this effect is found to be between 20 and 40 days of age. Interestingly, the period of sexual maturation in the rat (20 to 40 days) also corresponds to the maturation of the melatonin rhythm in the pineal gland (Tamarkin *et al.*, 1980; Reiter *et al.*, 1985). In this context, it is likely that neonatal pinealectomy could have consequential effects expressed at a later age than the immediate post-pubertal age. The few

studies on pinealectomy in rats have not probed the effect in the adult condition i.e. at 90 days or beyond. This becomes pertinent in the context of recently reported enlargement of testis and increased sperm production manifested much later in the adult stage caused due to transient neonatal hypothyroidism (Cooke *et al.*, 1991; Meisami *et al.*, 1992). Moreover, pineal has also been reported to have influence on thyroid and adrenal functions in birds and mammals (Virend *et al.*, 1979; Vaughan *et al.*, 1982; Virend, 1983; 1984; Patel *et al.*, 1985; Ramachandran and Patel, 1986).

Since, some of the above studies have indicated a subtle influence of pineal in the neonatal period, the present study has been designed to evaluate the effects of neonatal Px on the functional maturation of the male reproductive system and thyroid on a long term basis extending up to adult stage in the Charles-foster strain of rats.

RESULTS

I. MORPHOMETRIC OBSERVATIONS

BODY WEIGHT (Table 2.1; Fig.1 & 2)

The body weight was greater in the control rats at 35 days while that of the Px animals was lower. The control animals showed continuous increase in body weight from 35 to 90 days except for a slight decline between 35 and 60 days. The body weight at 90 days was significantly higher in Px rats than the control animals. On a percentage basis the increase in body weight between 35 and 90 days was more in Px rats than compared to the controls.

ORGAN WEIGHTS

Testes (Table 2.2 a, b; Fig. 3 a, b & 4)

The testes weighed significantly higher in Px rats at 35 days compared to the controls. The testes weight increased continuously and steadily between 35 and 90 days in control animals. The Px animals showed only marginal increase between 35 and 45 days, which was then

Table 2.1 Chronological alterations in Body Weight (gm), Percentage Difference and Per Day Growth Rate in intact and pinealectomised (Px) 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
Px	48.62 ± 4.43[b]	66.66 ± 3.09[d]	56.66 ± 4.44[d]	133.76 ± 10.02[a]	+ 37.10	- 15.00	+ 136.07	+ 175.13	1.39 ± 0.07[ns]	1.80 ± 0.12[d]	-	2.57 ± 0.20[d]

@ Values expressed as Mean ± SD of five experiments

[a] $p < 0.05$; [b] $p < 0.025$; [d] $p < 0.001$; [ns] Not Significant

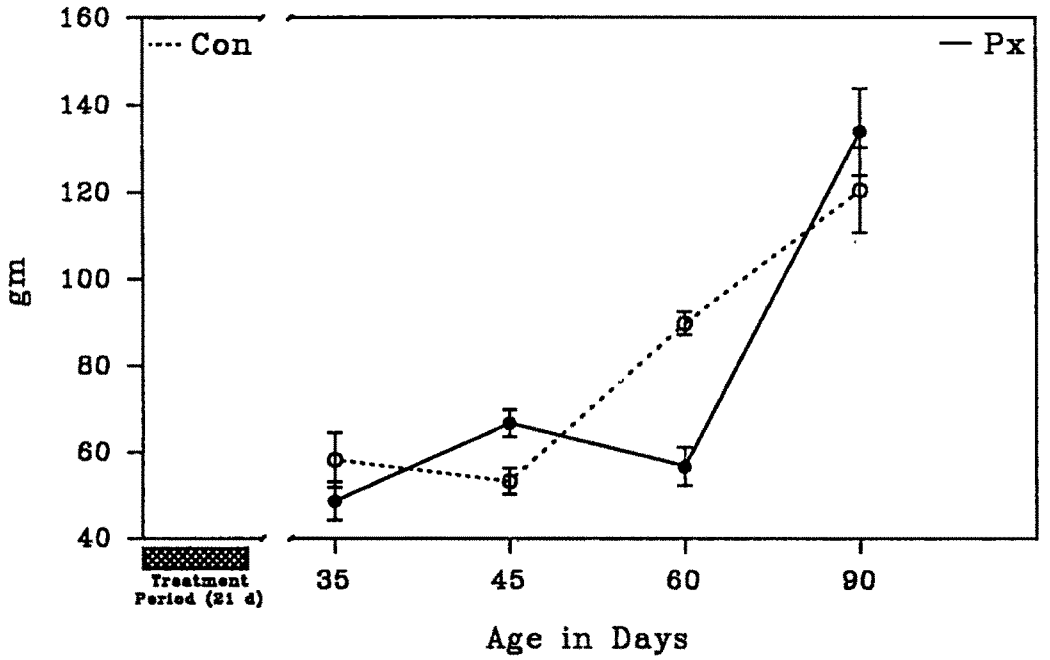


Fig. 1 Chronological alterations in body weight of neonatal rats subjected to pinealectomy (Px)

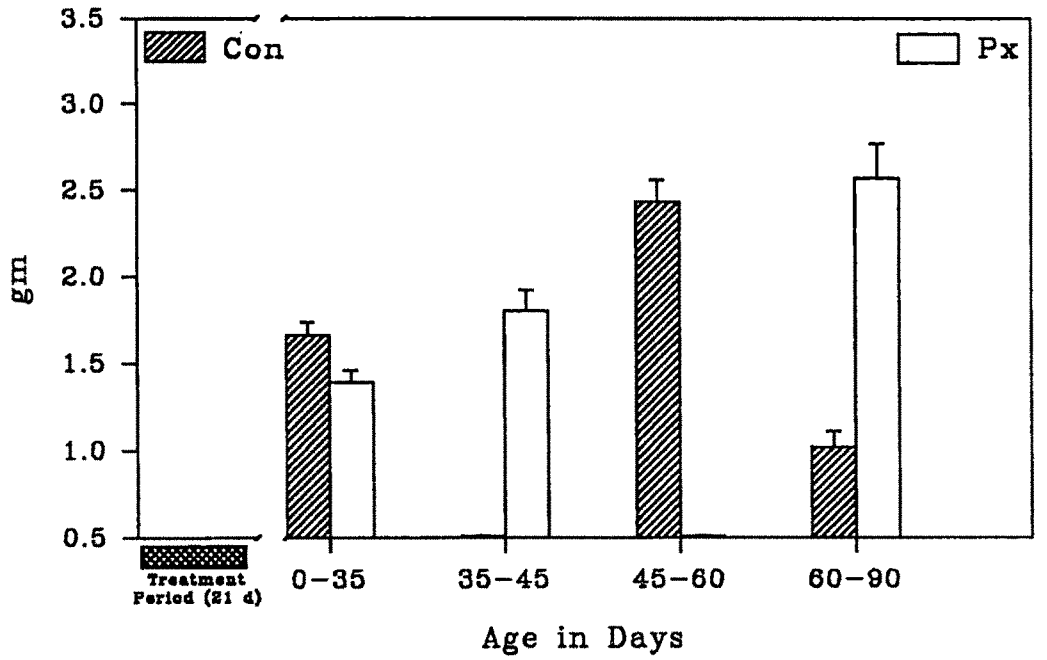


Fig. 2 Per day body growth in intact and pinealectomised rats

Table 2.2 (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 pinealectomised (Px) 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
	Px	585.60[d] ± 26.88	598.88[d] ± 23.03	1481.00[a] ± 61.78	2929.64[d] ± 36.49	1.21[d] ± 0.04	0.89[d] ± 0.02	2.62[d] ± 0.42	2.17[d] ± 0.04
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
	Px	96.40[c] ± 9.32	78.00[c] ± 6.28	188.6[d] ± 19.09	600.00[d] ± 27.21	0.19[ns] ± 0.02	0.12[ns] ± 0.01	0.33[d] ± 0.02	0.45[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
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
	Px	+ 2.27	+ 147.29	+ 95.99	+ 395.49	16.73[a] ± 1.53	1.33[d] ± 0.15	58.81[c] ± 4.98	47.35[c] ± 4.50
EPIDIDYMIS	Con	- 47.68	+ 513.56	+ 27.75	+ 310.09	3.51 ± 0.32	-	22.06 ± 6.45	3.65 ± 0.24
	Px	- 19.09	+ 141.89	+ 217.99	+ 522.41	2.75[ns] ± 0.28	-	7.38[d] ± 0.65	13.71[d] ± 1.02

@ Values expressed as Mean ± SD of five experiments; [a] $p < 0.05$; [c] $p < 0.01$; [d] $p < 0.001$; [ns] Not Significant

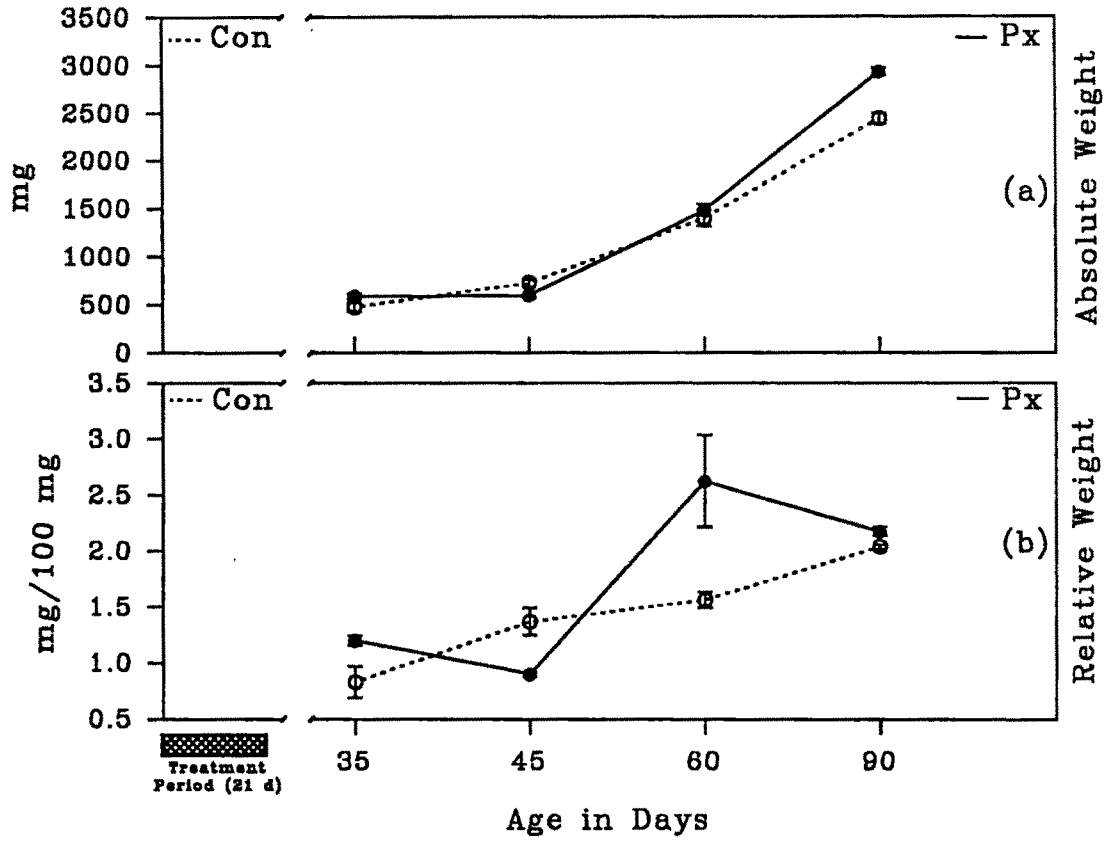


Fig. 3 (a&b) Chronological alterations in absolute and relative weights of testes in intact and pinealectomised (Px) rats

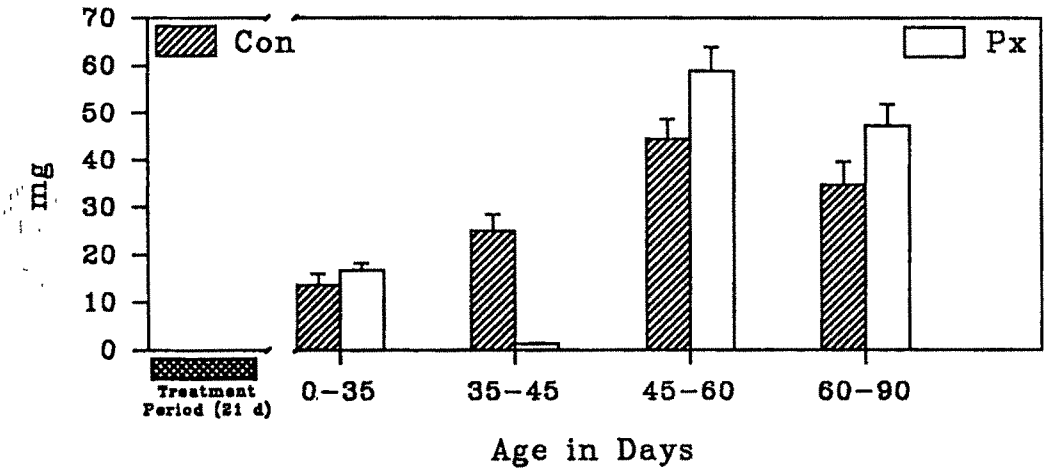


Fig. 4 Rate of growth of testes in intact and pinealectomised rats

followed by significant increase between 45 and 90 days. The final testes weight at 90 days was greater in Px animals than the control rats. In terms of relative weight also, the higher testes weight at 35 days was in Px animals. The relative weight of testes showed continuous increase in control animals between 35 and 90 days. The Px animals showed maximal relative weight at 60 days with a decrement at 45 and 90 days. However, the final weight at 90 days was more than the control rats. In the control animals, the per day growth rate was maximum between 45 and 60 days while the same was more pronounced in Px animals during that period. The control animals showed continuously and steadily increased growth rate between 35 and 90 days, whereas the Px animals depicted a drop in growth rate between 35 and 45 days, which was otherwise higher than the control at all periods.

Epididymis (Table 2.2 a, b; Fig. 5 a, b & 6)
The epididymis weighed significantly less than the controls in Px rats at 35 days. Both in control and Px animals, the weight of the epididymis decreased significantly at 45 days and was more pronounced in the former group of rats. Both control and Px animals showed continuous and significant increase in the weight of the epididymis between 45 and 90 days. The control animals showed the maximum growth spurt between 45 and 60 days, while the same occurred between 60 and 90 days in Px animals. The ultimate absolute weight of epididymis at 90 days was greater in Px animals compared to the controls. Relative weight of epididymis in both the groups of rat paralled the changes in absolute weight at all stages. Similar changes were noted in both the groups of rat on a percentage basis. In the control animals, the growth rate of the epididymis was maximum between 45 to 60 days with a period of not much growth between 35 and 45 days.

Seminal Vesicle (Table 2.3 a, b; Fig. 7 a, b & 8)
At 35 days, the absolute weight of seminal vesicle was almost similar in both control and Px rats. The weight of the seminal vesicle showed a decrease at 45 days in both control and Px rats and was more pronounced in the Px rats. Between 45 and 90 days, the weight of seminal vesicles

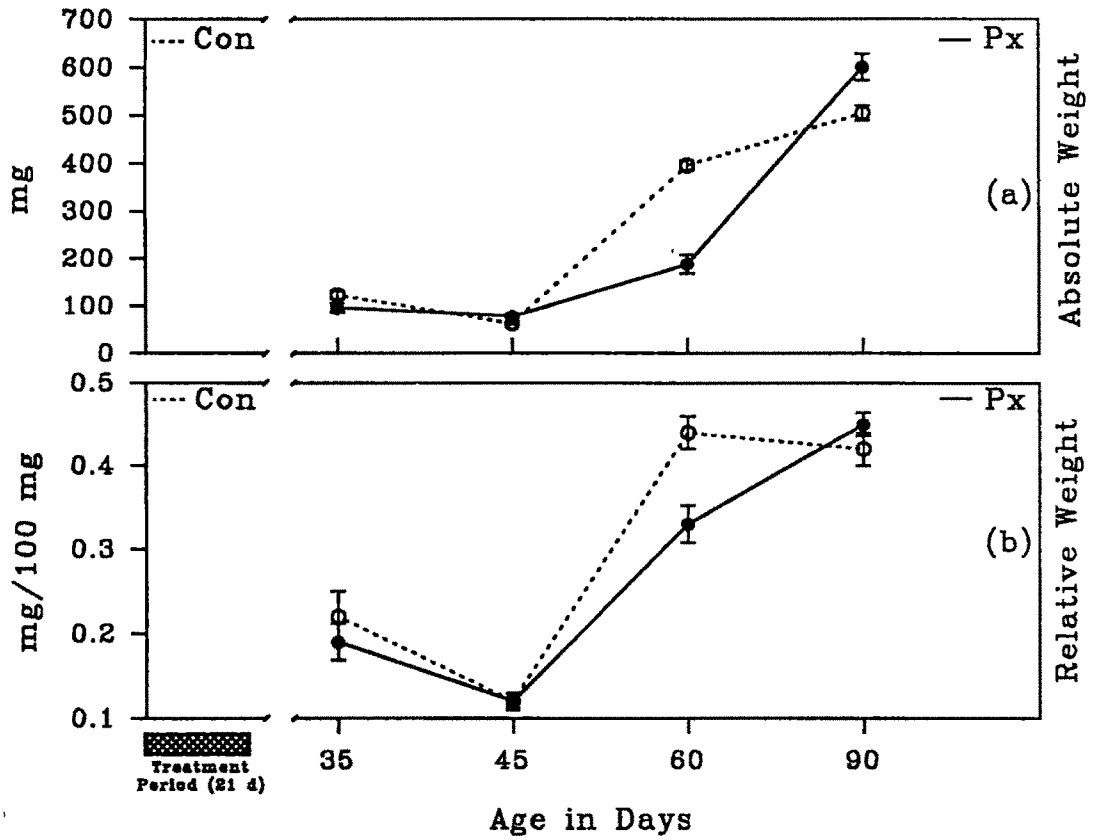


Fig. 5 (a&b) Chronological alterations in absolute and relative weights of epididymis in intact and pinealectomised (Px) rats

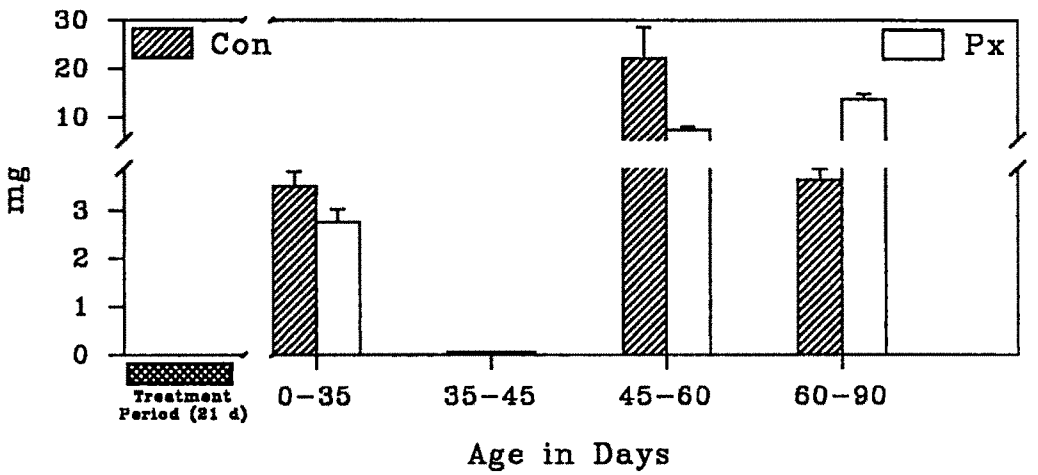


Fig. 6 Rate of growth of epididymis in intact and Px rats

Table 2.3 (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 pinealectomised (Px) 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
	Px	21.80^{ns} ± 3.57	13.84^{a} ± 4.98	95.07^{c} ± 10.21	646.06^{d} ± 24.40	0.044^{ns} ± 0.006	0.021^{d} ± 0.003	0.168^{d} ± 0.007	0.484^{d} ± 0.014
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
	Px	26.68^{ns} ± 2.34	25.42^{d} ± 4.27	22.08^{d} ± 2.26	189.00^{c} ± 13.94	0.054^{c} ± 0.005	0.038^{d} ± 0.002	0.039^{d} ± 0.004	0.141^{d} ± 0.006

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
	Px	- 36.51	+ 586.92	+ 581.67	+ 2872.75	0.62^{ns} ± 0.05	-	5.41^{c} ± 0.98	18.43^{d} ± 1.05
PROSTATE GLAND	Con	- 37.94	+ 435.51	+ 89.87	+ 531.03	0.69 ± 0.09	-	4.41 ± 0.95	2.43 ± 0.89
	Px	- 4.72	- 13.14	+ 755.98	+ 608.39	0.76^{ns} ± 0.04	-	-	5.56^{d} ± 0.99

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

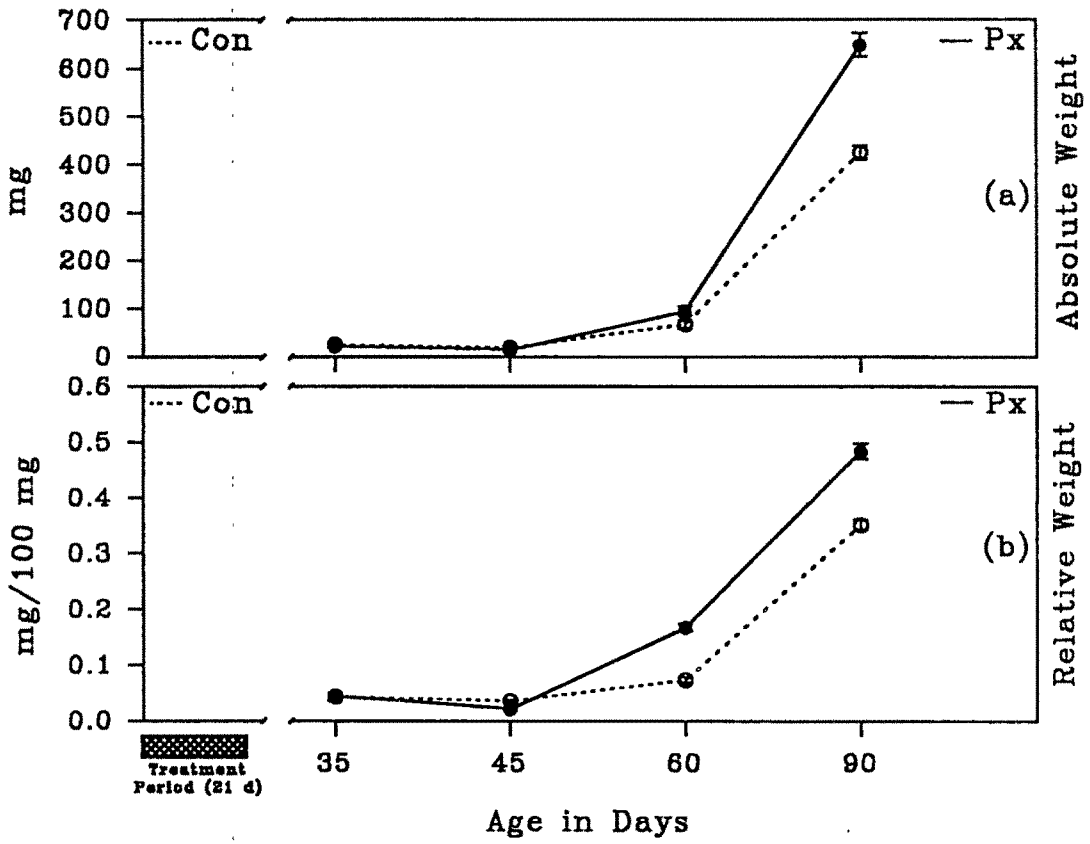


Fig. 7 (a&b) Chronological alterations in absolute and relative weights of seminal vesicle in intact and pinealectomised (Px) rats

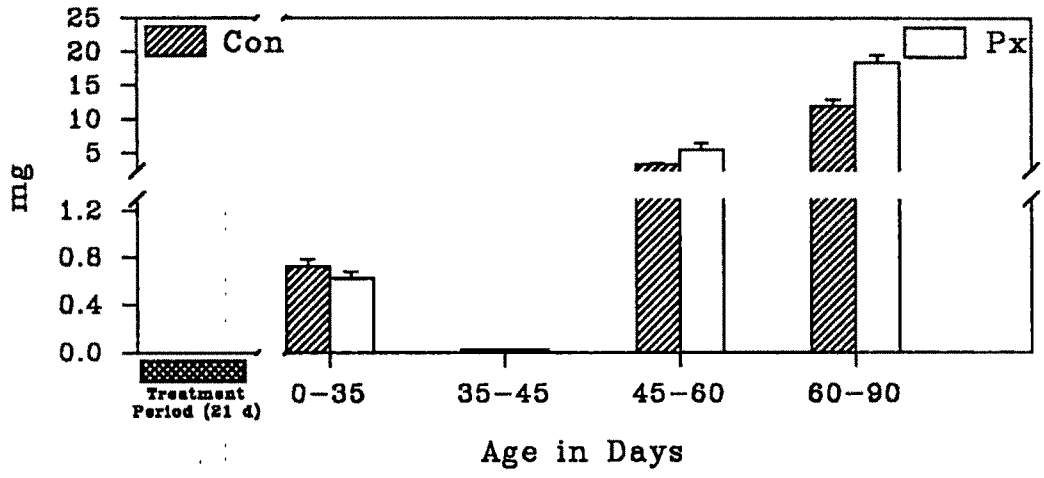


Fig. 8 Rate of growth of seminal vesicle in intact and Px rats

increased continuously and significantly in both control and Px group of rats. Whereas the maximum growth spurt occurred between 60 and 90 days in control animals, the growth rate remained the same between 45 and 60 and 60 and 90 days in Px animals. The final weight of the seminal vesicle at 90 days was greater in the Px animals compared to the control rats. The changes in the relative weight of seminal vesicle in both the groups at all the stages reflected the changes in the absolute weight. On a percentage basis, the maximum increase in seminal vesicle weight occurred between 60 and 90 days in the controls, while, the same was registered between 45 and 60 days in Px rats.

Prostate Gland (Table 2.3 a, b; Fig. 9 a, b & 10)
The weight of prostate gland was more or less similar in both control and Px animals at 35 days. There was a significant decrement in weight at 45 days in control animals. In the Px animals, the weight remained more or less constant between 35 and 45 days. In the control rats the weight of prostate gland increased continuously between 45 and 90 days with a maximal growth rate between 45 and 60 days and, the same was registered in Px animals between 60 and 90 days with a non-significant decrease in weight between 45 and 60 days. The ultimate weight at 90 days was significantly greater in Px animals compared to the controls. The relative weight of the prostate in both the groups of rat at all the stages reflected the changes in absolute weight. Similar trend in weight was depicted by both the groups at all stages on a percentage basis.

Thyroid Gland (Table 2.4; Fig. 11 a & b)
The thyroid weight was identical in both control and Px groups of rat at 35 days. At 45 days, there was significant decrement in the weight of thyroid in both the groups, the least being in the Px group. Between 45 and 90 days the thyroid weight increased in both control and Px rats. Finally the weight of the thyroid was identical in both control and Px animals at 90 days. The maximal weight gain in thyroid in control animals occurred between 45 and 60 days, while, the same occurred in Px animals between 60 and 90 days. The relative weight of thyroid in both the

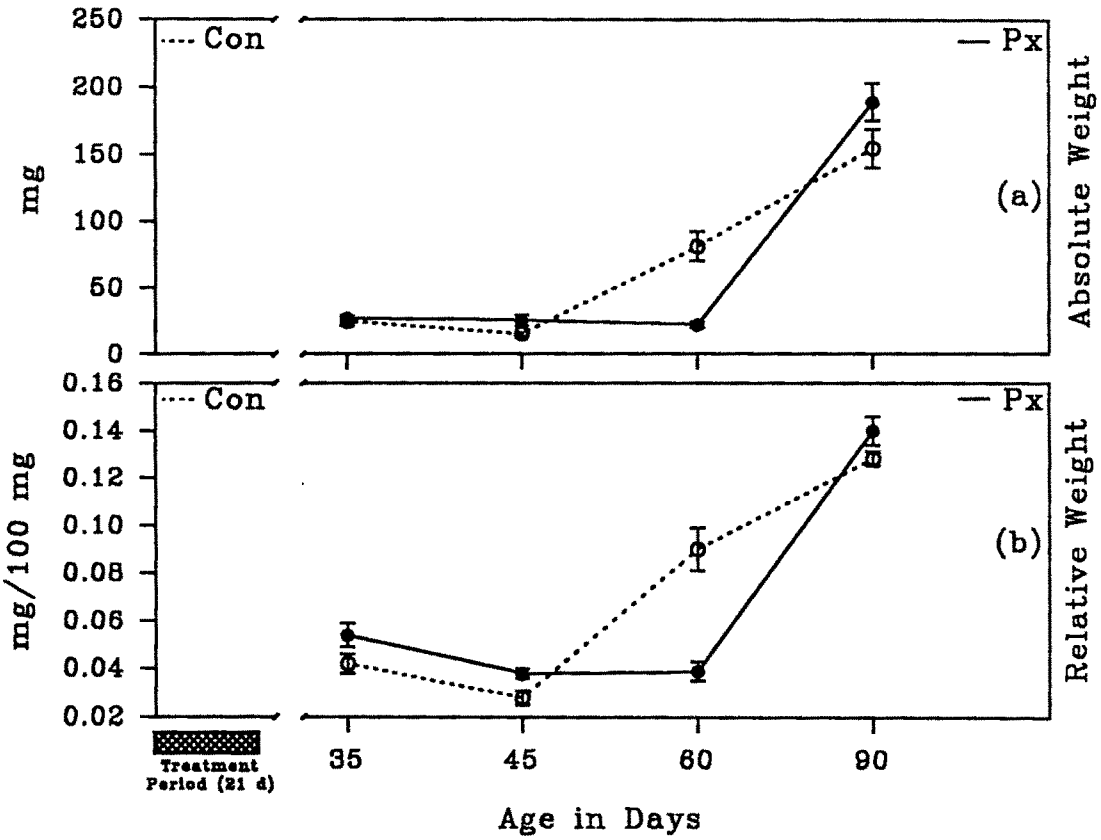


Fig. 9 (a&b) Chronological alterations in absolute and relative weights of prostate gland in intact and pinealectomised (Px) rat

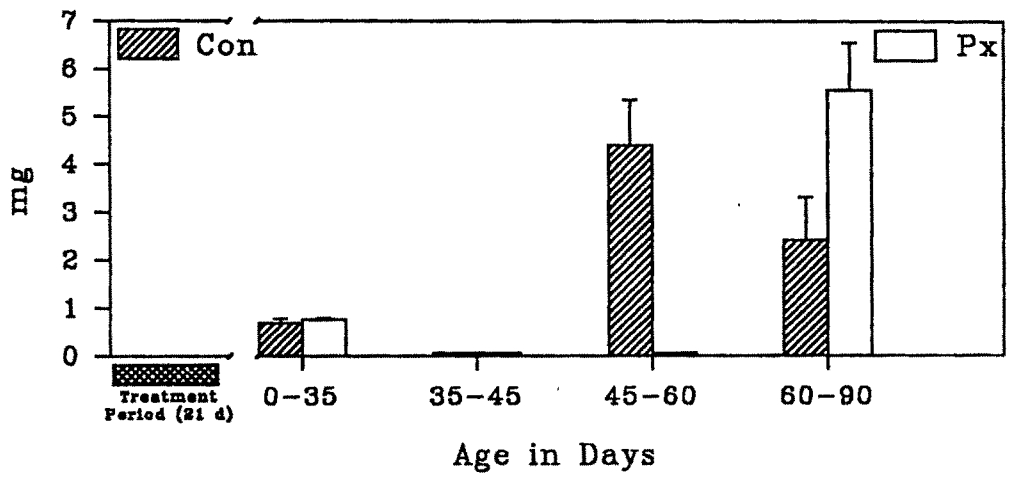


Fig. 10 Rate of growth of prostate gland in intact and Px rats

Table 2.4 Chronological alterations in Weight [Absolute (mg) and Relative (mg/100mg)] and Percentage Difference of Thyroid Gland in intact and pinealectomised (Px) 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	0.010 ± 0.002	0.006 ± 0.001	0.007 ± 0.0006	0.007 ± 0.0005	- 41.33	+ 88.07	+ 22.36	+ 35.009
Px	6.40 ± 0.55^{ns}	5.66 ± 0.82^{d}	5.22 ± 0.45^{d}	8.60 ± 0.95^{ns}	0.013 ± 0.001^{c}	0.009 ± 0.001^{d}	0.009 ± 0.0003^{d}	0.006 ± 0.0005^{c}	- 11.56	- 7.77	+ 64.75	+ 34.37

@ Values expressed as Mean ± SD of five experiments

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

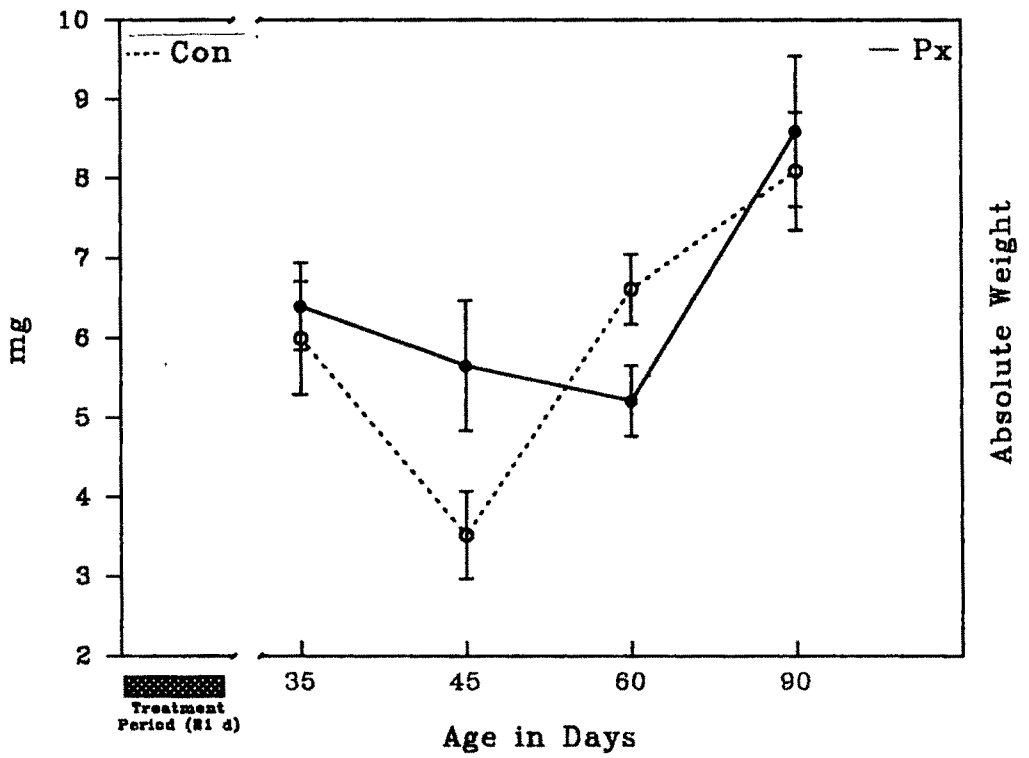


Fig. 11 a Chronological alterations in absolute weight of thyroid gland in intact and pinealectomised (Px) rats

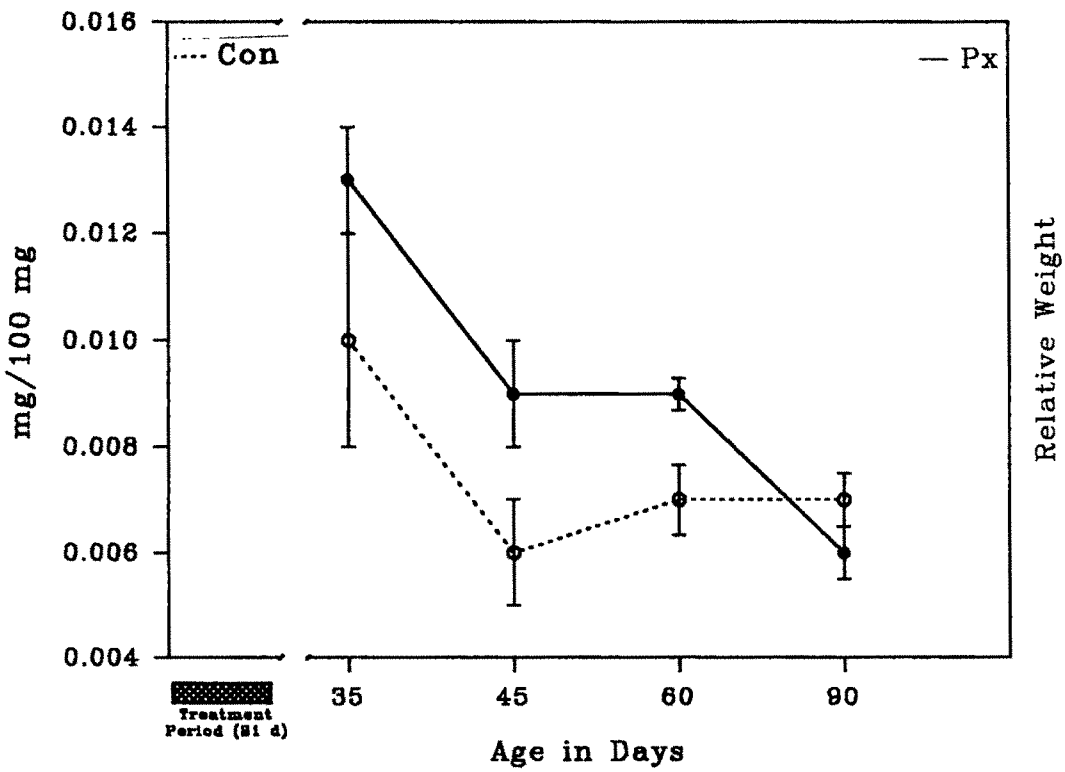


Fig. 11 b Chronological alterations in relative weight of thyroid gland in intact and pinealectomised (Px) rats

Table 2.5 Chronological alterations in the Diameter (in μm) of Seminiferous Tubule and Epididymis (Caput and Cauda) in intact and pinealectomised (Px) 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
Px	152.38 ± 11.33^{d}	120.95 ± 9.29^{ns}	149.81 ± 11.11^{a}	170.76 ± 14.30^{ns}	26.33 ± 1.62a	29.05 ± 2.83^{a}	29.86 ± 3.29^{ns}	34.18 ± 2.89^{d}	17.86 ± 1.24^{d}	18.28 ± 1.61^{c}	17.05 ± 1.80^{a}	16.76 ± 1.13^{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

groups was maximal at 35 days and more in Px animals. The relative weight of thyroid at 90 days was minimal for both the groups.

II. HISTOLOGICAL OBSERVATIONS

STRUCTURE OF TESTIS (Table 2.5; Plates I-III)

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.

Px: The tubules were significantly enlarged, with an average diameter of 152.38 μm. Compared to the controls, there were relatively more primary spermatocytes in the zygotene stage. Degenerating cells were less evident and overall, the germ cell population appeared to be quantitatively more. The interstitial cells were more prominent compared to the controls.

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.

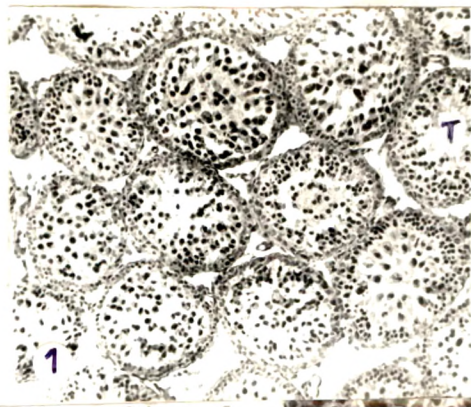
Px: The average diameter of the tubules was like in the control's (120.95 μm), but was much less as compared to the diameter of the tubules at 35 days of the Px animals. There was rampant degeneration of germ cells with many tubules showing degenerating cells in the center. The overall cell population appeared to be decreased. Pachytene spermatocytes could be seen though many meiotic germ cells seem to be undergoing degeneration. The interstitium appeared well formed.

PLATE I

Figures 1-6: Photomicrographs of sections of testis of 35 and 45 day old neonatally pinealectomised rats.

Figures 1(100 x) and 2,3(200 x): Sections of 35 day old rats showing enlarged tubules (T), prominent interstitium (I) and quantitatively more number of germ cells and spermatocytes (Sc). With very few degenerating germ cells (arrow).

Figures 4(100 x) and 5,6(200 x): Sections of 45 day old rats showing decreased germ cell population and increased spermatocyte degeneration (arrow). Interstitium (I) is prominent.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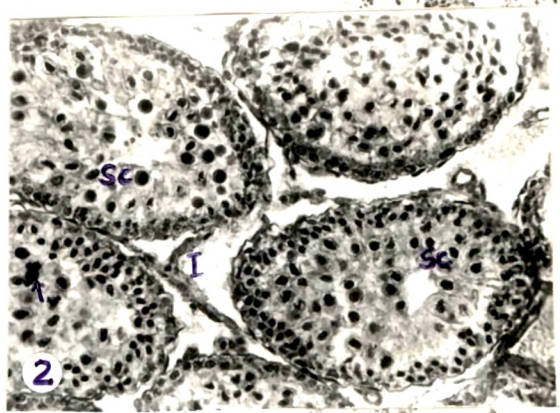
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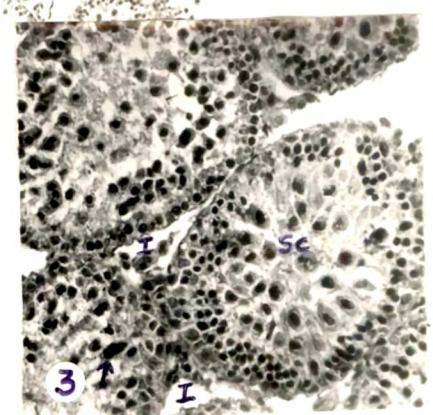
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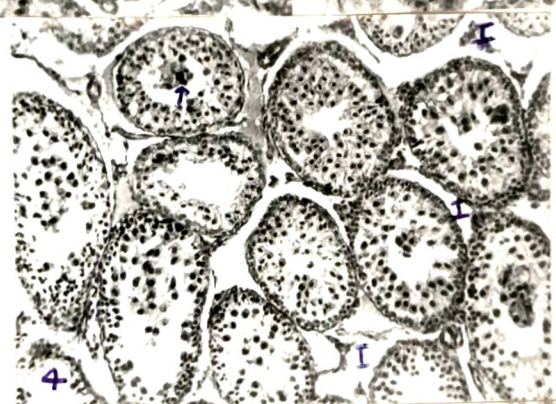
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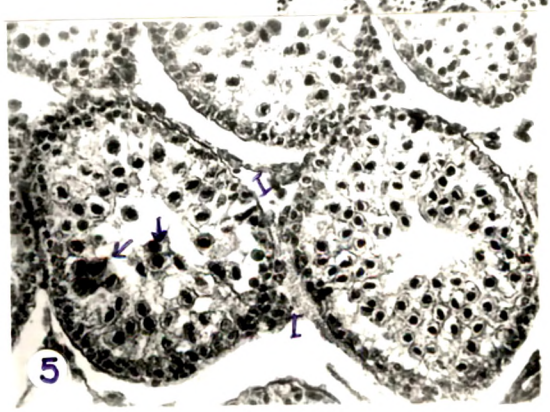
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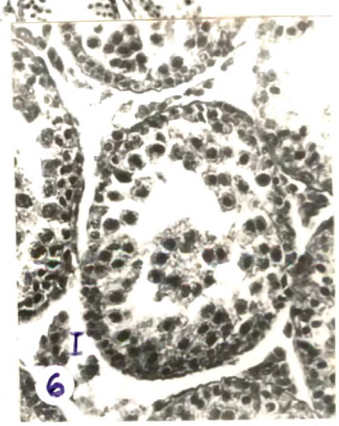
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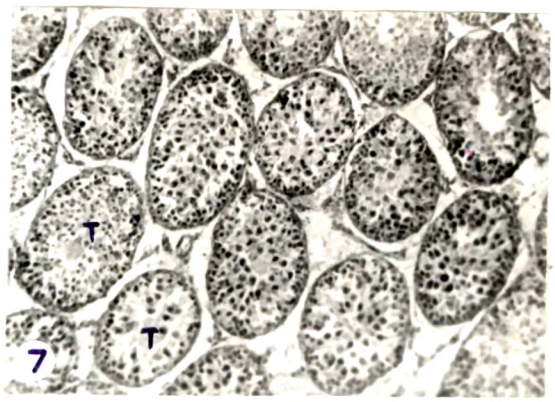
PLATE II

Figures 7-10: Photomicrographs of sections of testis of 60 day old neonatally pinealectomised rats (100 x- Figures 7,9 and 200 x- Figures 8,10).

Figures 7-10: Sections of 60 day old pinealectomised rat showing well formed tubules (T) and progression of spermatogenesis and appearance of round spermatids (RSt).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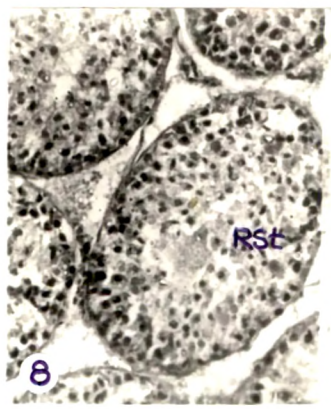
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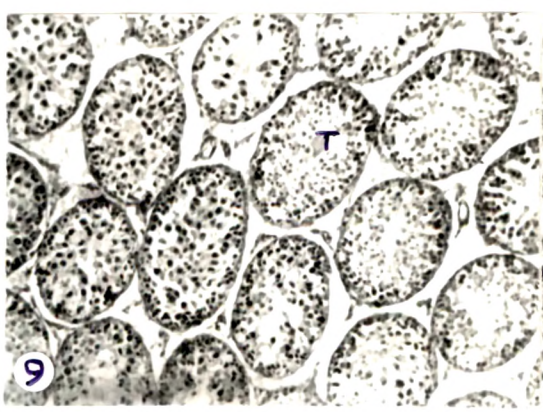
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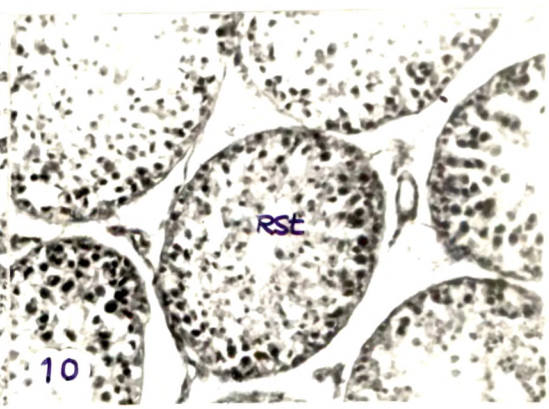
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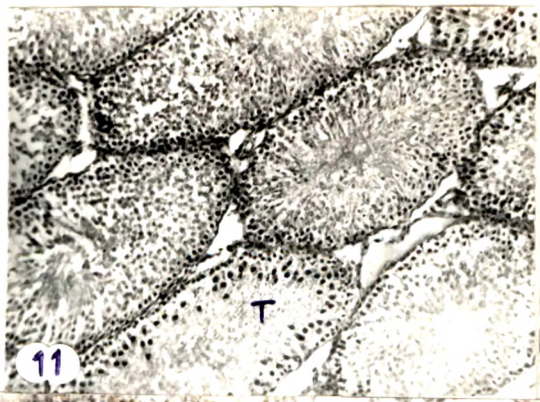
PLATE III

Figures 11-19: Photomicrographs of sections of testis of 90 day old neonatally pinealectomised rats (100 x- Figures 11,13 and 16 and 200 x- Figures 12,14,15,17-19).

Figures 11-19: Sections of 90 day old pinealectomised rats showing histological features of testis. Note the compactly packed tubules (T) with fully established spermatogenesis. Overall germ cell density appears more and spermatozoa (Sz) and spermatids (St) could be seen in many of the tubules. Interstitium (I) is well developed.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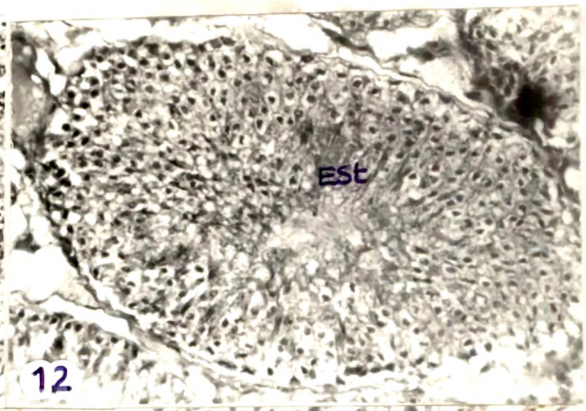
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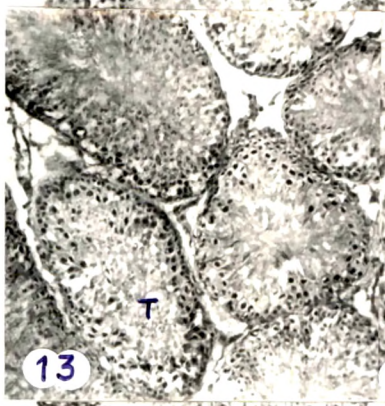
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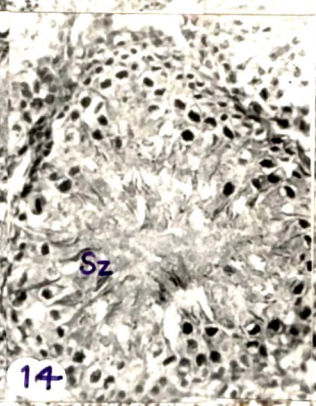
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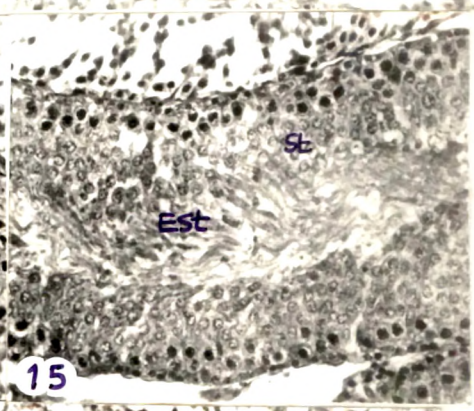
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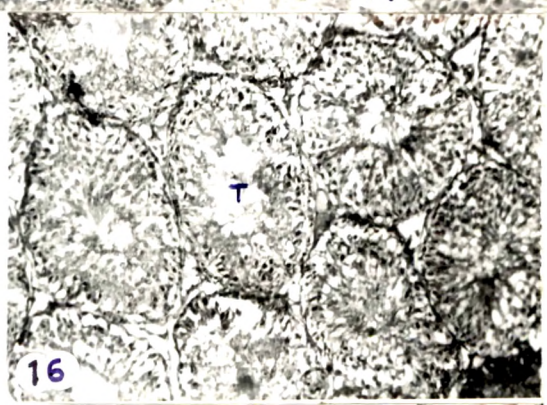
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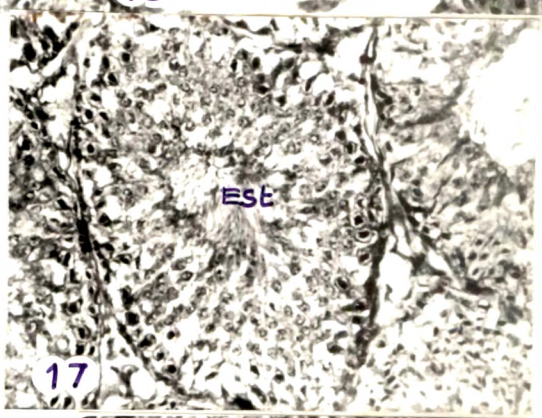
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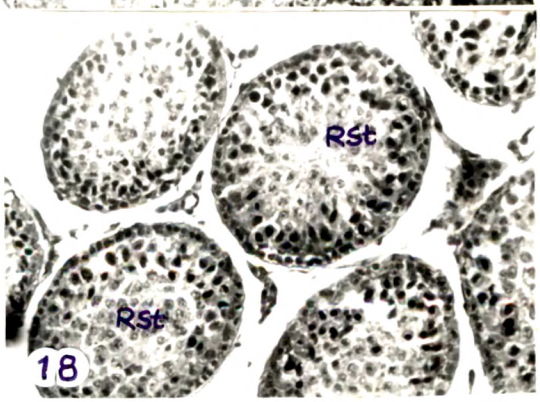
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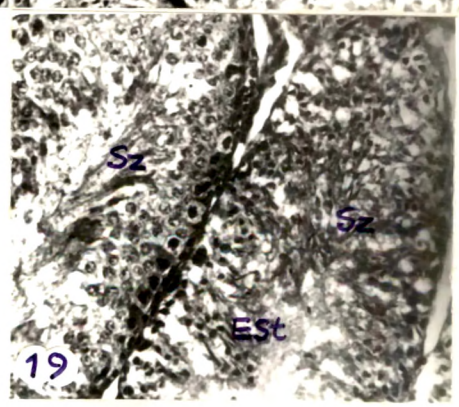
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60 Day Old

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.

Px: The tubules were enlarged as compared to 45 days with an average diameter of 149.81 μm, markedly less than the controls. However, the tubules were well formed and spermatogenesis had advanced up to round spermatids. 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 appeared to be moderately developed.

Px: The tubules increased in size with a diameter of 170.76 μm, less than the controls. They were compactly packed with spermatogenesis fully established with the tubules showing presence of spermatozoa. The sperm density in the tubules appeared to be more than in controls. Interstitium was moderately developed.

STRUCTURE OF EPIDIDYMIS (Table 2.5; Plate IV)

35 Day Old

Control: The tubules are 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

Px: Compared to controls, the tubules appeared slightly smaller with comparable cell height as that of controls. The lumen contained hardly any germ cells.

PLATE IV

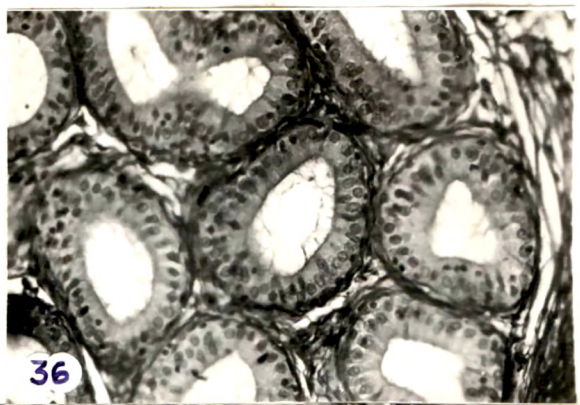
Figures 36-42: Photomicrographs of sections of epididymis of 35,45,60 and 90 day old neonatally pinealectomised rats (200 x).

Figure 36: Section of epididymis of 35 day old rat showing larger tubules than the controls with hypertrophied epithelium.

Figures 37 and 38: Sections of epididymis of 45 day old rats showing well developed compactly packed tubules with greater cell height. The lumen is strewn with degenerating germ cells.

Figures 39 and 40: Sections of epididymis of 60 day old rat showing large compactly packed tubules with prominent epithelium. Lumen contains some degenerating germ cells

Figures 41 and 42: Sections of epididymis of 90 day old rat showing well formed large tubules lined by tall columnar epithelial cells. The lumen contains round and elongating spermatids and few spermatozoa.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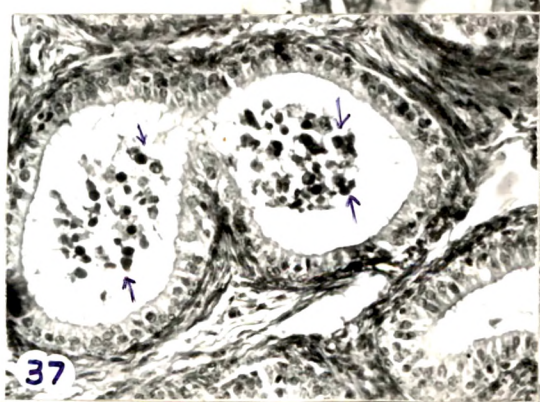
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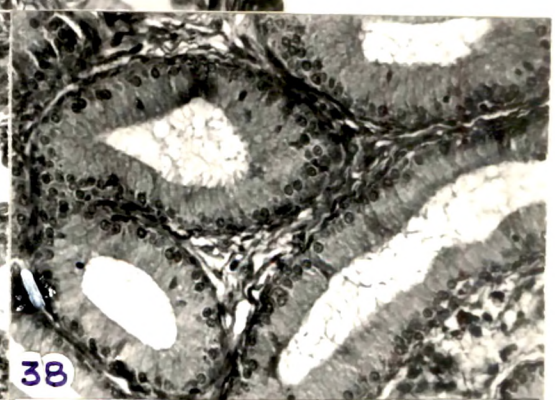
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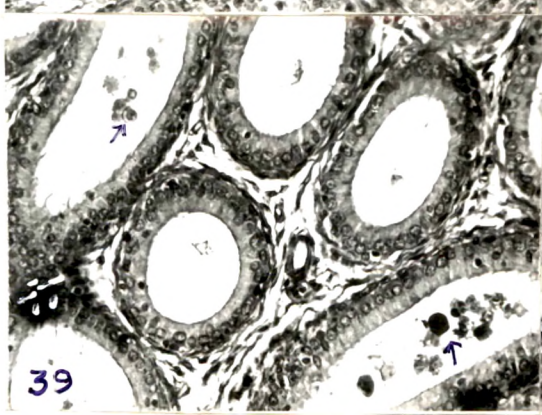
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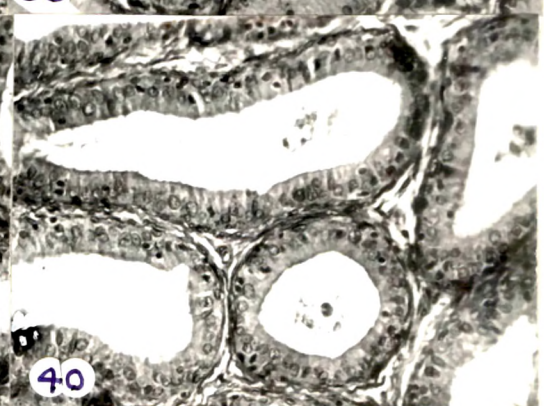
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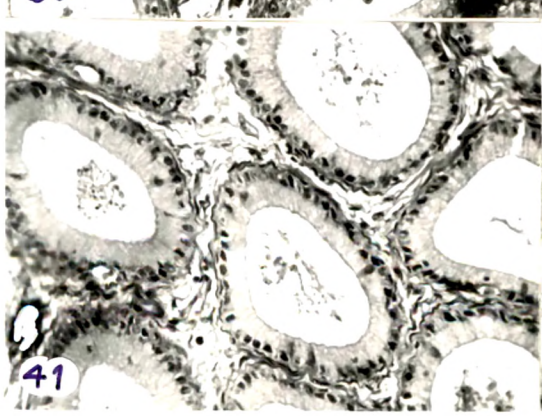
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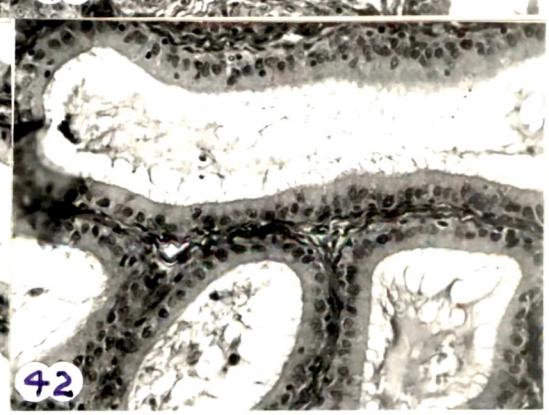

42

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.

Px: The tubules were well developed and compactly packed and appeared more convoluted. The cell height was greater than the control, ranging between 18.3 to 29.05 μm. The lumen was narrow and was strewn with 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.

Px: The epididymis was well developed with large compactly packed tubules. The epithelial cell height was not much different compared to 45 days and was less than that of 60 day control animals (17.05 to 29.8 μm). No sperms could be seen in the lumen though some degenerating germ cells were evident.

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.

Px: The tubules were large and well formed lined by columnar cells. The cell height was significantly less than the controls and ranged between 16.7 to 34.2 μm (obviously the cell height remained constant from 45 days onwards, where as the controls showed continuous increase from 35 to 90 days).

STRUCTURE OF SEMINAL VESICLE (Plate V)

35 Day Old

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

Px: The epithelium appeared hypertrophied and secretory material could be seen in the lumen

PLATE V

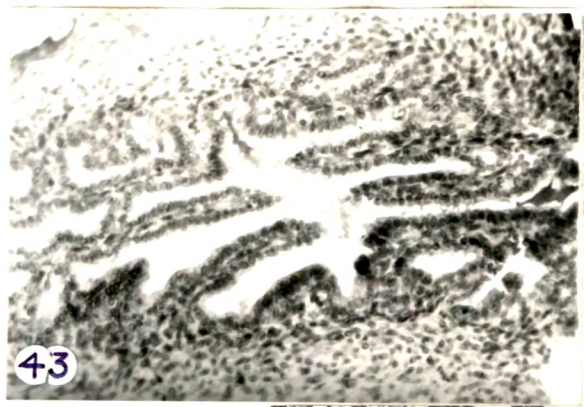
Figures 43-48: Photomicrographs of sections of seminal vesicle of 35,45,60 and 90 day old neonatally pinealectomised rats (200 x).

Figures 43 and 44: Sections of seminal vesicle of 35 day old rat showing secretory epithelium lined by hypertrophied cells and secretory material (S) in the lumen.

Figure 45: Section of seminal vesicle of 45 day old rat showing well developed secretory epithelium with prominent hypertrophied cells. Secretory material (S) is evident in the lumen .

Figure 46: Section of seminal vesicle of 60 day old rat showing prominent, convoluted secretory epithelium lined by columnar cells. Colloidal secretory material (S) could be seen in the lumen.

Figures 47 and 48: Sections of seminal vesicle of 90 day old rat showing prominent and convoluted secretory epithelium. Holocrine secretory activity clearly evidenced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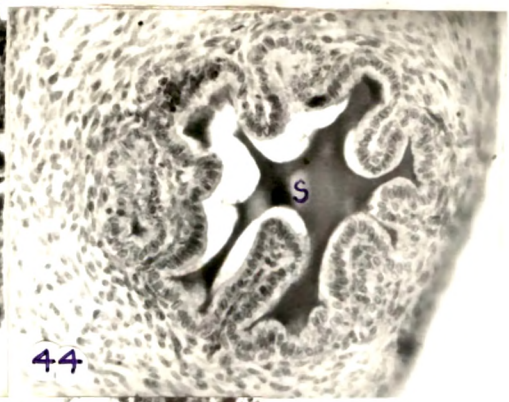
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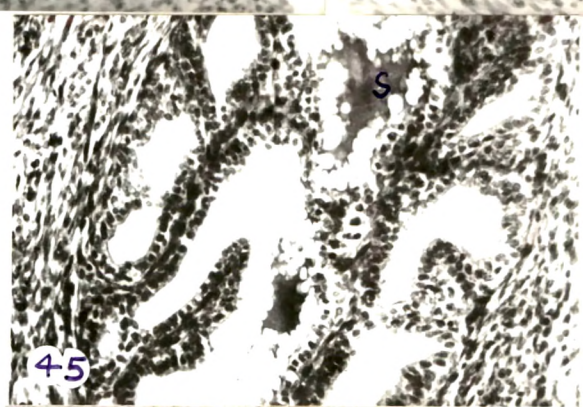
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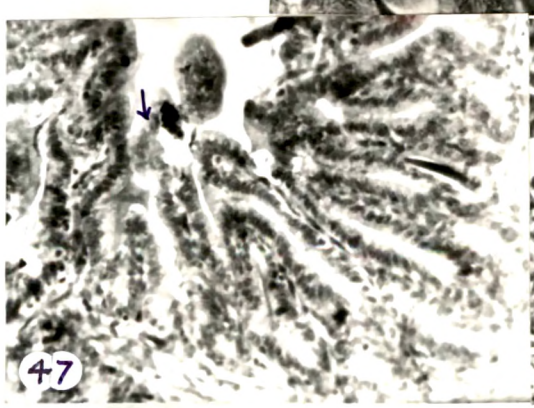
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48

45 Day Old

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

Px: The epithelium was well developed with hypertrophied cells. Secretory material could be seen in the lumen.

60 Day Old

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

Px: The epithelium was prominent and convoluted lined by hypertrophied cells. The lumen was narrow and contained colloidal secretory material.

90 Day Old

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

Px: The secretory epithelium was prominent, well developed, convoluted and more like the controls.

STRUCTURE OF PROSTATE (Plate VI)

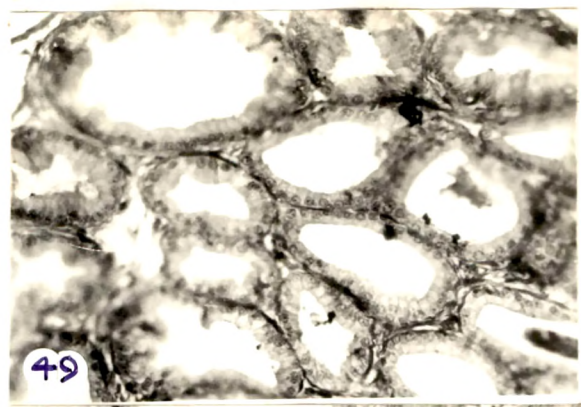
35 Day Old

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

Px: The acini were better developed than the controls and more convoluted.

45 Day Old

Control: The acini were well developed and convoluted and lined by tall columnar epithelium. The epithelium also appeared pseudostratified.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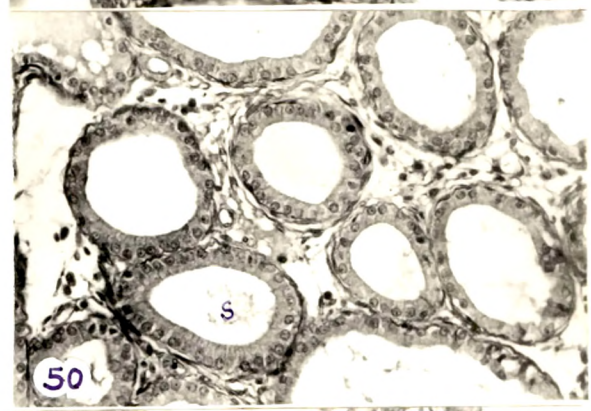
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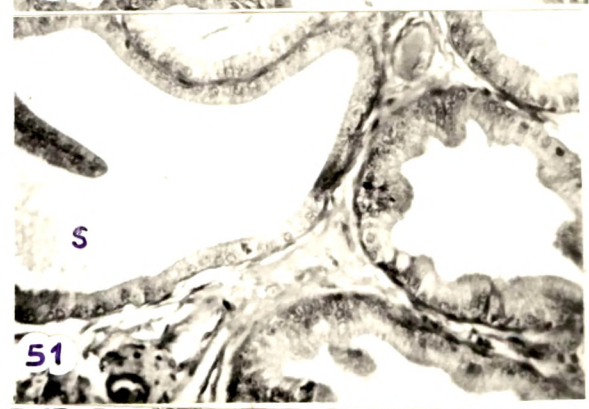
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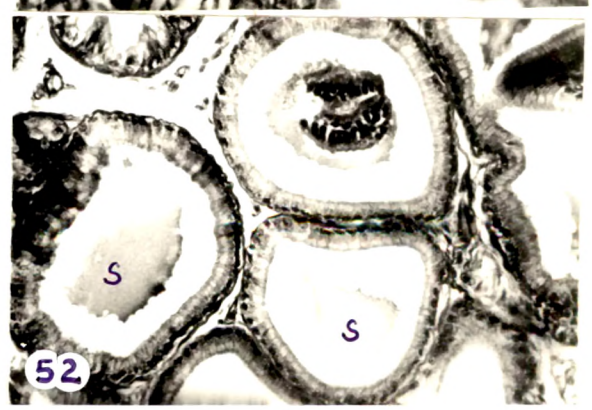
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Px: The acini were well formed, convoluted and lined by tall columnar epithelium. The lumen contained amorphous secretory material.

60 Day Old

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

Px: The histological appearance was very much similar to that seen at 45 days.

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.

Px: The acini were large and convoluted and lined by cuboidal to columnar epithelium. The lumen was filled with abundant secretory material.

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.

Px: The thyroid appeared very active with the follicle cells greatly hypertrophied and the lumen containing very little colloid.

45 Day Old

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

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

60 Day Old

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

PLATE VII

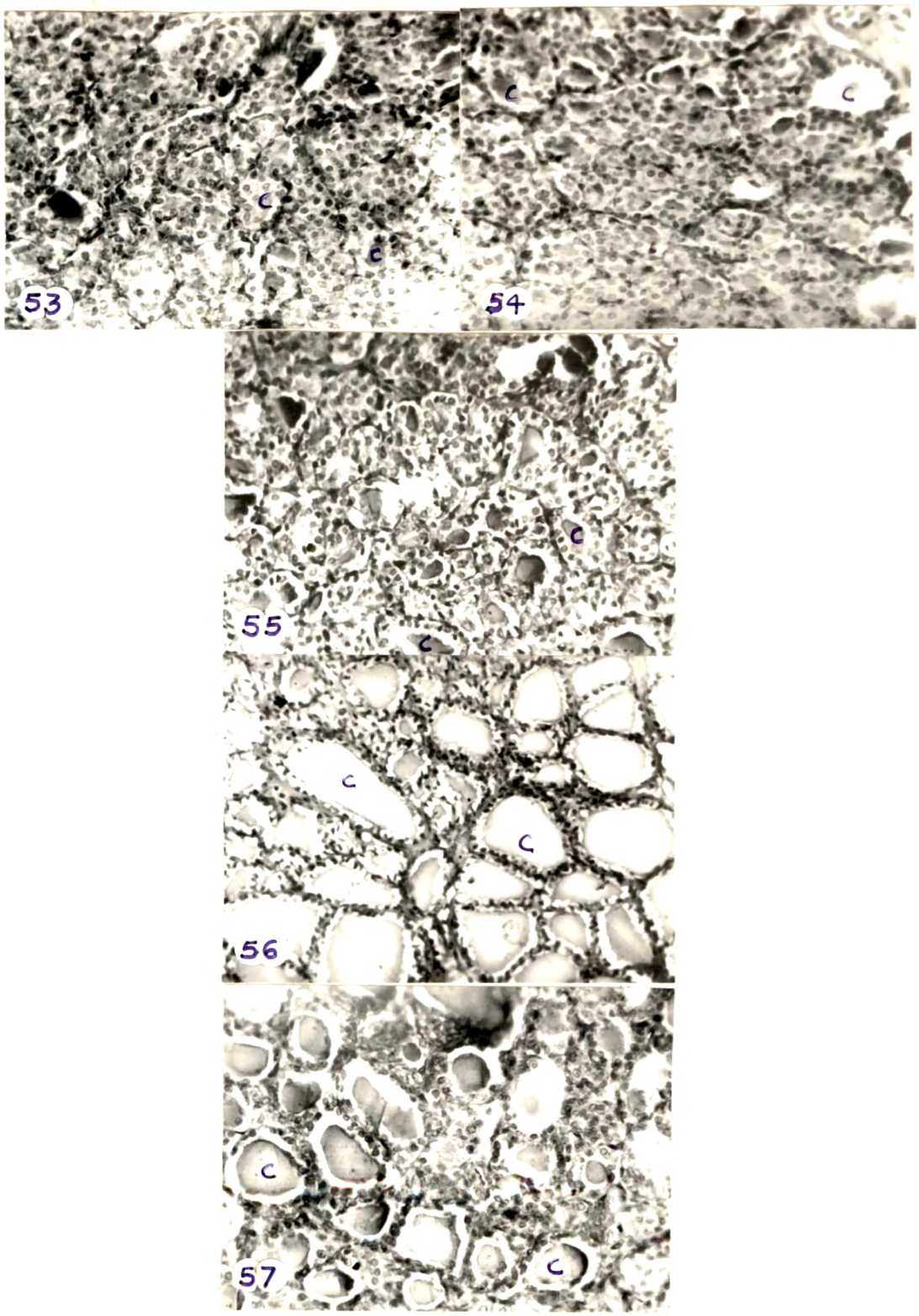
Figures 53-57: Photomicrographs of sections of thyroid of 35,45,60 and 90 day old neonatally pinealectomised rats (200 x).

Figures 53 and 54: Sections of thyroid of 35 day old pinealectomised rats showing active follicles lined by hypertrophied cells and with very little colloid (C) in the lumen .

Figure 55: Section of thyroid of 45 day old rat showing follicles with hypertrophied vacuolated epithelium and narrow lumen with little colloid (C) .

Figure 56: Section of thyroid of 60 day old rat showing well formed large follicles lined by cuboidal epithelium and filled with colloid (C) .

Figure 57: Section of thyroid of 90 day old rat showing follicles lined by high cuboidal epithelium with varied colloid content (C).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Px: The follicles were lined by cuboidal epithelium and most of the follicles appeared filled with colloid.

90 Day Old

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

Px: The follicles were lined by high cuboidal epithelium with some follicles appearing filled with colloid and some empty.

III. HISTOCHEMICAL OBSERVATIONS (Plates VIII & IX)

35 Day Old

3ß-HSDH: In the testis of control animals, the enzyme 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. In the Px animals, there was increase in enzyme activity in the Leydig cells, more pronouncedly visible with DHEA as the substrate. The enzyme activity in the tubules was week with both the substrates.

17ß-HSDH: The enzyme activity was appreciable in the tubules while no activity was visible in the Leydig cells of control rats. Appreciable enzyme localization was discernable in the tubules with no activity in the Leydig cells of Px rats.

3*α*-HSDH: The enzyme activity was weakly localised in the tubules but not in the Leydig cells of control rats. In the testis of Px rats the tubules appeared enzyme positive, while the Leydig cells showed negative response.

45 Day Old

3ß-HSDH: 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 enzyme activity in the Leydig cells with DHEA as substrate which was not evident with P. In general in Px rats, the enzyme activity was more than in the control testis with the Leydig cells

PLATE VIII

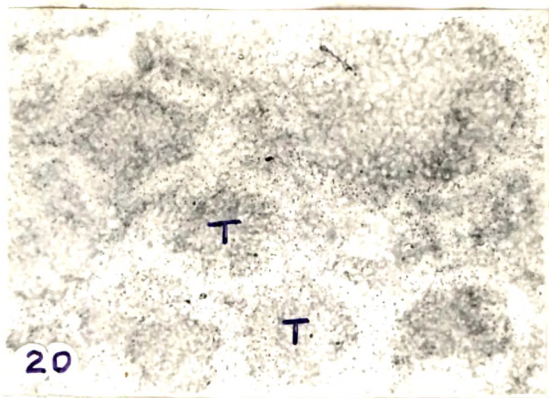
Figures 20-27: Photomicrographs of sections of testis of 35,45,60 and 90 day old neonatally pinealectomised rats showing histochemical localisation of 3α and 17ß HSDH (65 x).

Figures 20 and 24: Sections of 35 day old rat showing 3α and 17ß HSDH activity respectively. Note the weak localisation of both the enzymes in the tubules (T) and weak activity of 17ß HSDH in the interstitium (I).

Figure 21 and 25: Sections of testis of 45 day old rat showing 3α and 17ß HSDH localisation respectively. Note the strong 3α HSDH activity in the interstitium (I) with feeble activity in the tubules (T) (Fig. 21) and noticeable activity in the interstitium (I) and feeble activity in the tubules (T) for 17ß HSDH (Fig.25).

Figures 22 and 26: Sections of testis of 60 day old rat showing 3α and 17ß HSDH localisation respectively. Fig.22 showing noticeable to moderate activity in the interstitium (I) and very little activity in the tubules (T) of 3α HSDH. Fig.26 showing some activity of 17ß HSDH activity in the periphery of the tubules (T) and almost no activity in the interstitium.

Figures 23 and 27: Sections of testis of 90 day old rat showing 3α and 17ß HSDH localisation respectively, showing intense activity of both 3α and 17ß HSDH activity in the tubules (T) and weak activity of 3α HSDH (Fig.23) and noticeable activity of 17ß HSDH (Fig.27) in the interstitium (I).



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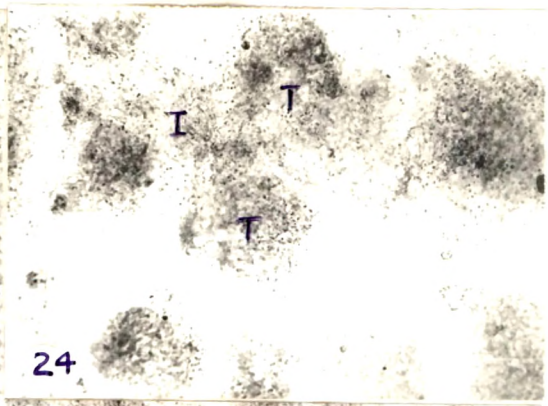

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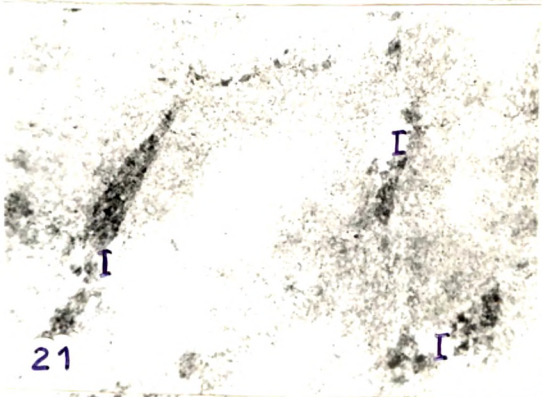

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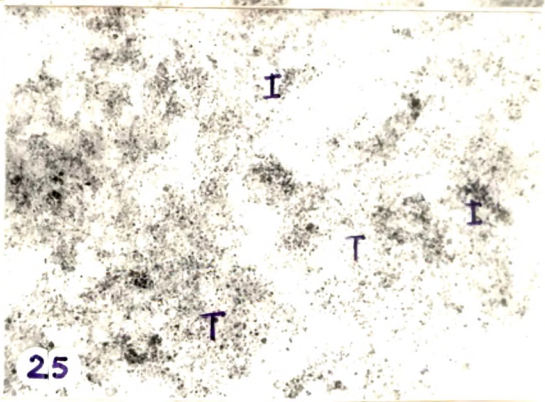

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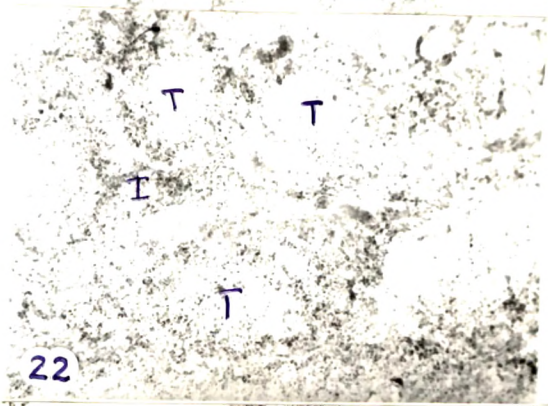

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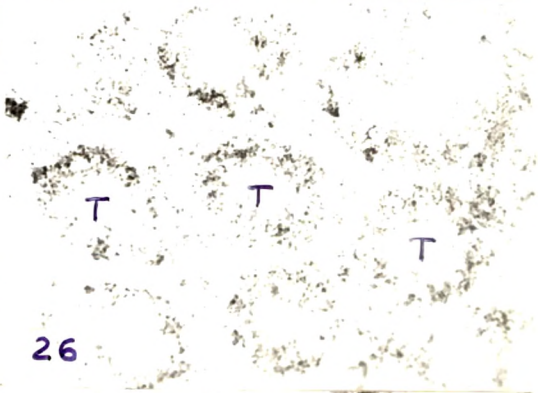

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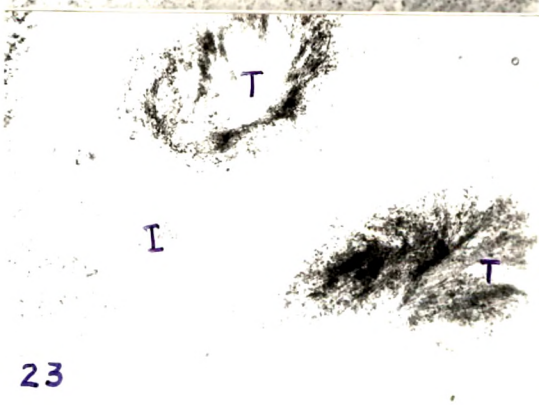

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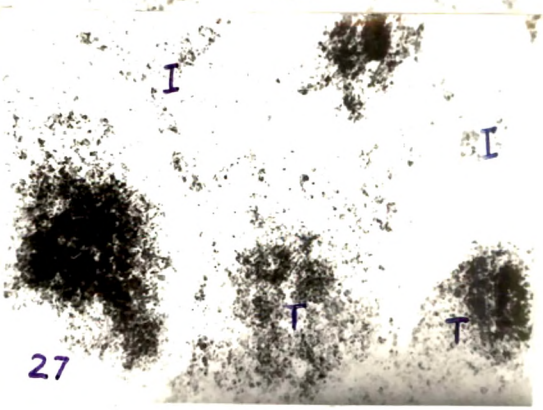

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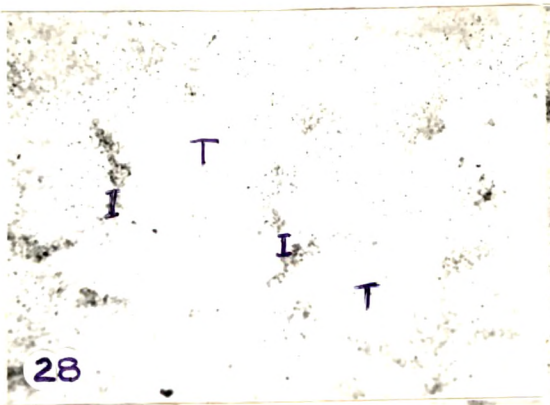

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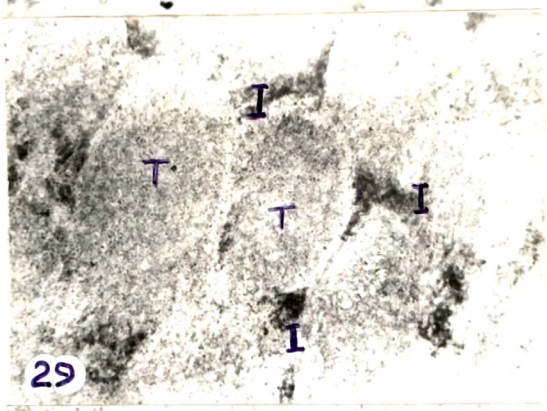

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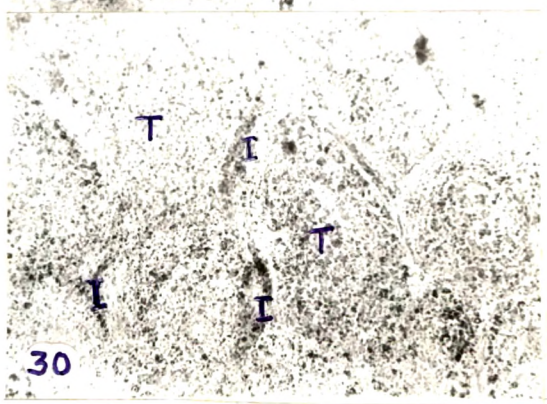

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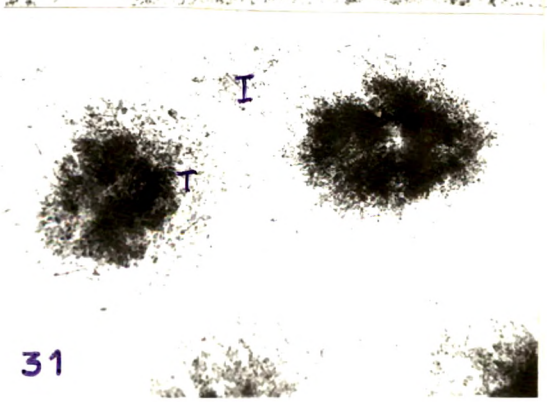

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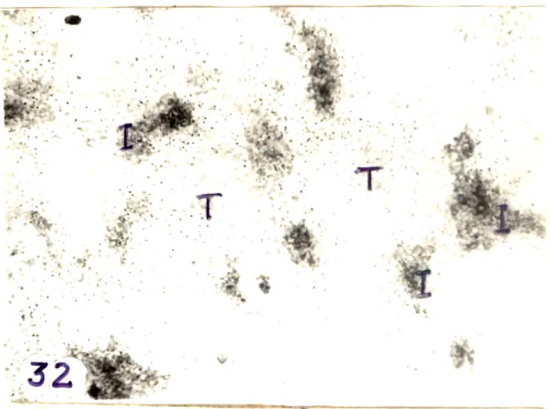

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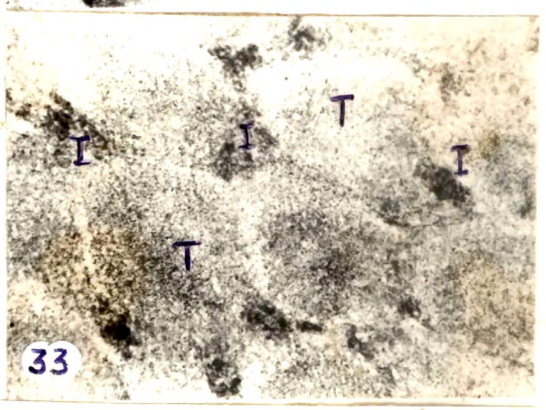

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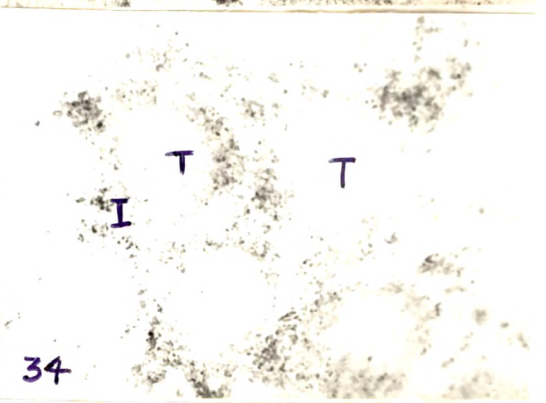

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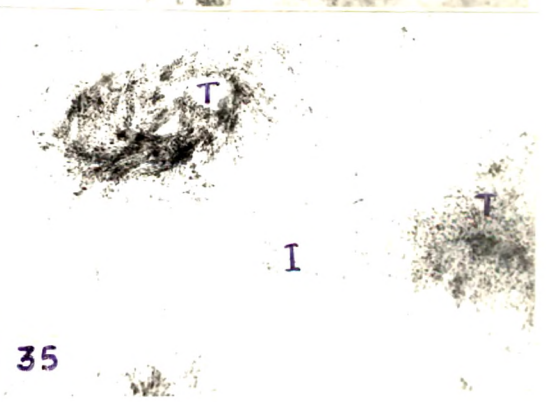

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PLATE IX

Figures 28-35: Photomicrographs of sections of testis of 35,45,60 and 90 day old neonatally pinealectomised rats showing histochemical localisation of 3ß HSDH with P and DHEA as substrates (65 x).

Figures 28 and 32: Sections of testis of 35 day old rat showing positive 3ß HSDH response in the interstitium (I) more prominent with DHEA as the substrate (Fig.32) than with P (Fig.28).

Figures 29 and 33: Sections of testis of 45 day old rat showing strong activity in the interstitium (I) and noticeable activity in the tubules (T) with both the substrates. Overall, the enzyme activity appears more prominent with P as the substrate (Fig.29).

Figures 30 and 34: Sections of testis of 60 day old rat showing noticeable activity in the interstitium (I) with DHEA as the substrate (Fig.34) and moderate activity with P (Fig.30). The tubules (T) show noticeable activity more uniform with P and peritubular with DHEA.

Figures 31 and 35: Sections of testis of 90 day old rat showing weak activity of 3ß HSDH with both the substrates in the interstitium (I) and intense activity in the tubules (T) containing advanced stages of germ cells, more prominent with P as the substrate (Fig. 31) as compared to DHEA as the substrate (Fig.35).

being relatively more enzyme reactive than the tubules. The enzyme activity in the tubules was nevertheless less than the controls. The response was more or less similar with both DHEA and P as substrates.

17ß-HSDH: In the control animals, the Leydig cells were weakly enzyme 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. In the Px animals, both the interstitium and tubules were enzyme positive. The interstitial cell response appeared to be more than the controls.

3α-HSDH: The enzyme activity was mild though discernible in tubules as well as in the interstitium of the control animals. The enzyme activity was clearly discernable in the tubules while, no activity was evident in the interstitium in Px animals.

60 Day Old

3ß-HSDH: The enzyme 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. In the Px animals, the interstitium was enzyme positive with DHEA as the substrate while, the tubules were more positive with P as the substrate. Overall, the enzyme activity in the tubules was week and was more like the 35 day controls.

17ß-HSDH: In the control rats, the tubules were more enzyme responsive than the interstitium, though the latter was also enzyme responsive. Compared with 3ß- HSDH, the enzyme activity was less at the same age. The enzyme activity was clearly discernable in the tubules as well as in the interstitium in the Px animals but was lesser than the controls.

3α-HSDH: In the control, the enzyme activity was very strongly localised in the tubules, almost as intense as 3ß- HSDH activity. The enzyme activity was moderate in the interstitium and week in the tubules in the Px animals.

90 Day Old

3ß-HSDH: In the controls, the enzyme 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. In Px animals, the enzyme activity was mild in the interstitium with both DHEA and P as substrates. The tubules were more enzyme responsive, prominently with P as the substrate. The enzyme activity was clearly discernible in the tubules containing the advanced stages of germ cells rather than tubules containing earlier stages.

17ß-HSDH: The enzyme activity was very strong and clearly discernible towards the luminal part containing advanced stages of germ cells in the testis of control animals. The interstitial cells were mildly enzyme responsive. Relatively, the enzyme activity was more than that of 3ß-HSDH. In the Px rats, the enzyme activity was less than the controls and more clearly distributed in the luminal part of the tubules containing advanced stages.

3α-HSDH: In the control animals, the enzyme activity was very much reduced as compared to 60 days and the intensity and distribution was similar to that of 3ß-HSDH. In Px rats, the enzyme activity was strongly localized in the tubules, more towards the luminal part containing advanced stages of germ cells.

IV. SERUM HORMONE PROFILE (Table 2.6; 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

Table 2.6 Chronological alterations in Serum Triiodothyronine, Thyroxine and Testosterone levels in intact and pinealectomised (Px) 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
Px	1.26 ± 0.04^{d}	2.79 ± 0.09^{ns}	3.62 ± 0.10^{c}	3.04 ± 0.09^{b}	33.96 ± 3.44^{d}	54.57 ± 4.18^{d}	79.60 ± 3.99^{d}	80.10 ± 5.16^{d}	1.22 ± 0.26^{d}	3.46 ± 0.41^{d}	0.91 ± 0.24^{ns}	0.92 ± 0.22^{c}

@ Values expressed as Mean ± SD of five experiments

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

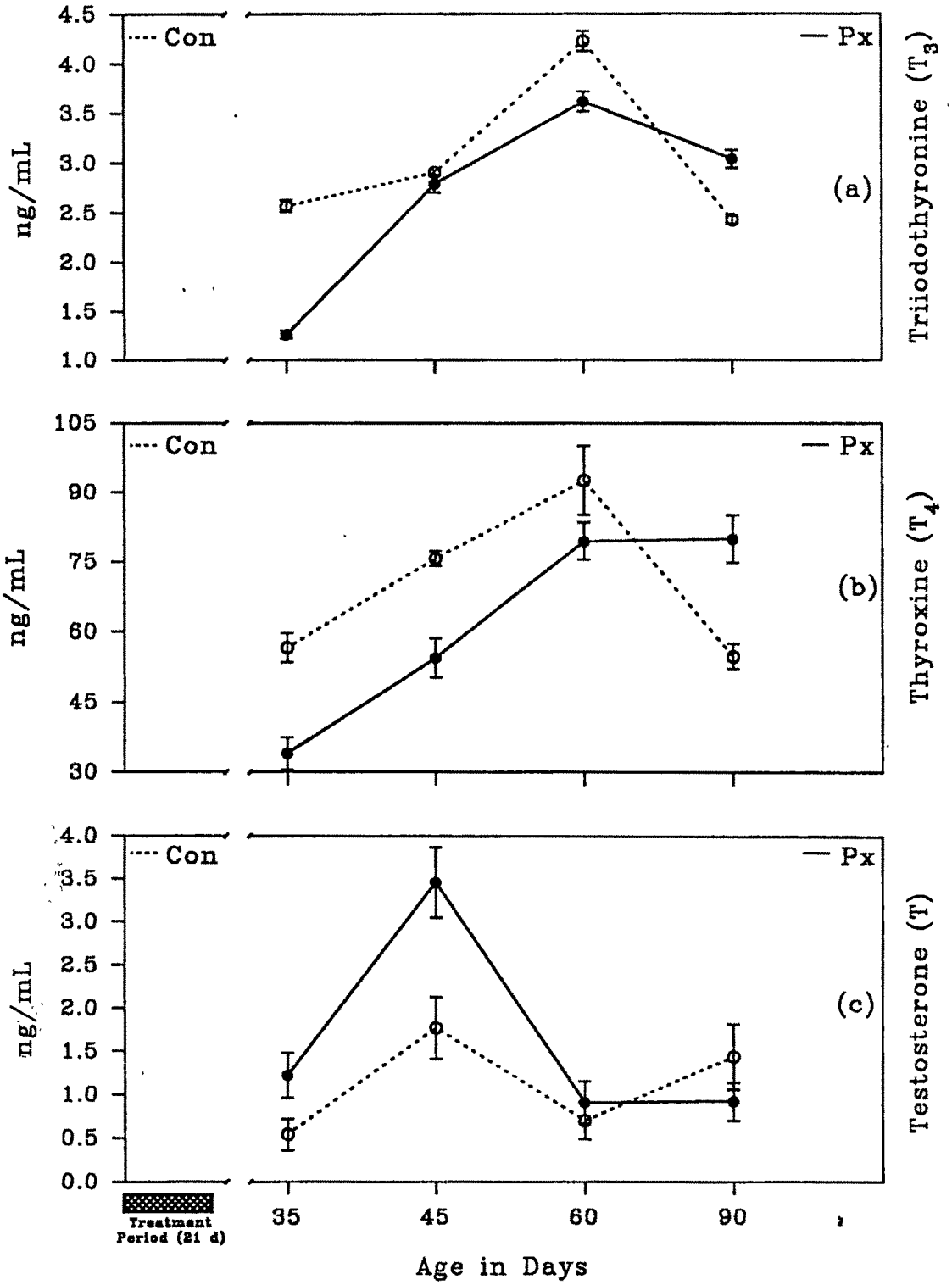

Fig. 12 (a, b & c) Chronological alterations in serum T_3, T_4 and T levels in intact and pinealectomised (Px) rats

days and then decreased by 90 days to the 35 day level. The serum T_3 and T_4 levels were significantly low in the Px rats at 35 days. Their levels increased thereafter to control levels by 45 days and then attained peak levels by 60 days. The levels tended to remain constant between 60 and 90 days with the result ultimately at 90 days the T_3 and T_4 levels in Px rats were significantly greater than those of the controls.

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. The serum T level was significantly greater in the Px rats from 35 to 60 days with the peak level at 45 days. The pattern of changes appeared to be similar to that of controls.

DISCUSSION

The influence of pineal on the reproductive system is well marked and more clearly understood in recent times in mammals with seasonal breeding cycles (Reiter, 1981; Arendt, 1988; Ebling and Foster, 1989; Stankov and Reiter, 1990). In contrast, the role of the pineal in the laboratory rat is not clearly elucidated, despite the fact that number of studies have been carried out. There are reports of varying effects of either Px or melatonin (Mel) administration in adult male rats, but the general consensus has veered to a concept of a no major role for pineal (Reiter, 1980; Goldman *et al.*, 1981; Binkly, 1983). Pinealectomy in neonatal or prepubertal rats was reported to be ineffective though, hastening of puberty has been noted (Motta *et al.*, 1967; Pitis and Maya, 1969; Kinson, 1976). However, Px combined with anosmia, food deprivation or neonatal testosterone (T) administration has been reported to have more marked effects (Reiter, 1981). Most of the earlier studies involving neonatal or prepubertal Px were restrictive in nature as the postoperative observations were confined to either the prepubertal or the pubertal period (Kinson, 1976; Reiter *et al.*, 1985).

In the present study, observations made with reference to the long term effects of Px have revealed delayed hypersensitivity response of the male reproductive system. Comparison of the body weights of control and Px animals between 35 to 90 days shows that Px animals weighed heavier at 45 and 90 days. However, at 35 and 60 days they weighed less than the controls. Some retardatory influence of Px is indicated by the chronologically observed changes in the growth kinetics. Whereas the control animals depicted a decrease in body weight, though less significant, the same occurred in Px animals between 45 and 60 days and was more marked than the controls. The maximum increase in body weight after the lag phase occurred in control animals between 45 and 60 days amounting to 69% increase and a per day growth rate of 2.43 gm. In Px rats, this increase occurred between 60 and 90 days amounting to 136%. Considering this pattern of changes it is very likely that the Px rats would still weigh heavier in the later periods.

The testes of Px animals weighed significantly heavier at 35 days. An early observation from this laboratory had also shown increased testes weight at 26 days in rats subjected to chemical Px by pCPA treatment in the pre-weanling period (Patel and Ramachandran, 1993). However, at 45 days, the weight of the testes of Px animals was significantly less than that of the controls, which was due to a static effect on gonadal growth between 35 and 45 days. After 45 days the Px animals showed significantly increased growth rate of testes resulting in 20% heavier testes at 90 days. The increased testes weight is paralleled by the increased weights of epididymis, seminal vesicles and prostate in Px animals. Like the body weight, the weight of the accessory organs also showed a decrement between 35 and 45 days in the control rats. This pattern was evident in the Px animals as well. Whereas the epididymis and prostate weighed approximately 20% heavier, the seminal vesicle weighed 53% heavier in Px rats at 90 days. In the control rats, while the epididymis and prostate showed maximum growth rate between 45 and 60 days, the seminal vesicles grew maximally between 60 and 90 days. In comparison in the Px

rats, whereas the epididymis and prostate grew maximally between 60 and 90 days, the seminal vesicle showed equally greater growth rate between 45 & 60 and 60 & 90 days.

The histological observation depicted larger tubules with more germ cells in the testis of Px animals at 35 days. However, between 35 and 45 days, there was a decrease in tubular diameter and reduced number of germ cells. Evidently, large scale degeneration of germ cells seen at this period seems to be the reason. But by 60 days there was recovery and reestablishment of spermatogenesis and sperms could be seen in the tubules at 90 days. In the control animals, spermatogenesis was fully established by 60 days with the appearance of sperms. In the Px rats, histological evidence indicates greater number of germ cells, though the diameter of the tubules was lesser than the controls at 90 days. The changes obtained in the present study appeared to be akin to those reported to be caused due to transient neonatal hypothyroidism (Cooke and Meisami, 1991; Meisami *et al.*, 1992; Kimberly *et al.*, 1993; Hess *et al.*, 1993). But neonatal hypothyroidism in Charles-foster strain did not produce the changes reported by the above workers (chapter 1), and this was accredited to an obvious strain difference. The major difference was with reference to the response of the Sertoli cells to hypothyroidism. Whereas hypothyroidism resulting in reduced T_4 and T_3 levels could prolong the period of Sertoli cell proliferation beyond 20 days in the Long-Evans and Sprague-Dawley strains of rats (Cooke and Meisami, 1991; Cooke *et al.*, 1992; Kirby *et al.*, 1992; Van Haaster *et al.*, 1992; Hess *et al.*, 1993), such an effect was not apparently shown by the Charles-foster strain (chapter 1). In this strain of rat, Px seems to exert the extended proliferative effect on Sertoli cells. The factors responsible for the increased Sertoli cell proliferation seemed to be a fortuitous combination of increased FSH level and decreased thyroid hormone level during the crucial window of Sertoli cell proliferation, i.e., the first 3 to 4 weeks of neonatal period. The levels of thyroid hormones and FSH seen in the Px animals at 35 days provide justification to this. Increase in serum gonadotropin levels consequent to Px is natural as pineal through its hormone melatonin is known to exert a suppressive effect on the hypothalamo-hypophysial-gonadal axis

(Kinson and Robinson, 1970; Kinson and Peat, 1971; Reiter, 1980; Lang *et al.*, 1984). This effect of melatonin is now clearly shown to be on the hypothalamic LHRH pulse generator which is exerted more powerfully in the prepubertal period (Silman, 1991). In this context, decreased serum gonadotropin levels consequent to melatonin administration as well as increased gonadotropin levels post Px or after exposure to long photic schedules have been shown to occur in both immature and mature animals (Binkly, 1983; Reiter *et al.*, 1985; Ebling and Foster, 1989).Though melatonin has been shown to have a suppressive effect on the hypothalamo-hypophysial-thyroid axis, leading to reduced serum thyroid hormone levels in both mammals and birds (Linda, 1981; Patel, 1993; Ramachandran *et al.*, 1996), in the present study, neonatal Px paradoxically produce a hypothyroid state in the prepubertal period. This is reflected in the observed low titres of thyroid hormone levels at 35 days and their gradual recovery to normal levels by 60 days. Obviously, the pineal has a positive influence on the hypothalamic-pituitary-thyroid axis in the neonatal period as against a negative influence in the adult condition. It is significant to note that the establishment of the pineal-melatonin rhythm and, the commencement of the maturation of the hypothalamo-pituitary-thyroid axis, both occur, between the first and second neonatal week (Dubois and Dussault, 1977; Balemans *et al.*, 1978). The maturation of the thyroid axis marked by increase in the pituitary TSH concentration and serum T_4 and T_3 levels and, higher melatonin levels both, coincidently occur by 3 weeks (Dussault and Labrie, 1975; Kieffer *et al.*, 1976; Dubois and Dussault, 1977; Pang *et al.*, 1984). The present observations seem to be incriminating in a sense Px retards the maturation of the hypothalamo-pituitary-thyroid axis and thereby contributing to an apparent hypothyroid state in the prepubertal to pubertal period.

The increased serum gonadotropin levels and reduced T_4 levels in the peripubertal period seemingly exerts different effects on the testes as reflected by the present observations. The increased serum LH levels consequent to Px is denoted by the increased Leydig cell number and their histochemical reactivity towards 3ß- and 17ß-HSDH and the serum T levels in the 35 and

45 old rats . The increased serum FSH hastens the initiation of spermatogenic process by stimulating spermatogonial proliferation as evidenced by the increased tubular size and germ cell population at 35 days. Another significant effect of increased FSH is the precipitating effect on the Sertoli cell proliferation. Such an influence of FSH was presumed earlier from studies on hemicastration induced testicular hypertrophy (Cuningham *et al.*, 1978) and has now been well established by the experimental administration of FSH during the critical window of Sertoli cell proliferation lasting up to 20 days (Meachem *et al.*, 1996). The above study showed that neonatal administration of FSH up to day 10 or day 15 increased the number of Sertoli cells by 18% and 49% respectively and increased germ cell numbers and overall testicular hypertrophy by 14% or 39% respectively at 90 days. They also observed an actual increase in the volume and length of seminiferous tubules with no effect on the diameter. Our present observations of increased testes weight by 20%, increased germ cell population and no increase in tubular diameter tally with the above and substantiate our concept that Px induced hypertrophy of adult testes is essentially due to the hyperproliferative effect induced by FSH in the first 3 weeks of neonatal period.

Considering the increase in testicular weight at 35 days in the Px rats, which was 122% of the controls, there was a distinct possibility of a much greater testicular hypertrophy than what was realised at 90 days. The attenuated hypertrophic response seen at 90 days could be related to the inhibited growth rate with a more or less plateaued testes weight between 35 and 45 days. Significantly this phase coincided with the period of extensive germ cell degeneration and a decrease in tubular diameter. This appears to be a consequence of the hypothyroid state. This is confirmed by previous observation of increased germ cell degeneration lasting up to 45 days due to neonatal hypothyroidism (Chapter 1). However, the absence of germ cell degeneration at 35 days when the HPOT state was more pronounced needs an explanation. Evidently germ cell proliferation and survival seen in the present study are supported by the increased FSH level. The requirement for FSH in this context is validated by the previous observation of

decreased serum FSH level and increased germ cell degeneration in animals subjected to neonatal hypothyroidism. Recent studies have implicated thyroid hormone in inducing differentiation and maturation of the Sertoli cells (Palmero *et al.*, 1989; 1995). The synergistic action of T_3, FSH and T is known to regulate Sertoli cell differentiation in the period subsequent to Sertoli cell proliferation (Bremner *et al.*, 1994; Varuberger *et al.*, 1994). In the Px rats, due to the subnormal/suboptimal thyroid hormone level, it is likely that the Sertoli cell differentiation is delayed. Due to the delayed differentiation and maturation of the Sertoli cells, the increased germ cells generated under the influence of FSH fail to find the conducive and supportive association with the Sertoli cells rendering them vulnerable to degenerative changes. The increased production of T in the Px rats, as evidenced by the histochemical observations as well as the serum LH and T levels, though conducive for spermatogenesis (Panno *et al.*, 1996), paradoxically appears contributing to germ cell degeneration due to the temporal delay in the expression of differentiated functions of Sertoli cells. Related events in this context are: **1** delayed expression of androgen receptors and increased expression of estrogen receptors in the Sertoli cells by reduced thyroid hormone levels (Panno *et al.*, 1996), and **2** greater sustained expression of aromatase activity by the immature Sertoli cells due to FSH action (Dorrington *et al.*, 1978; Van der Molen *et al.*, 1981). This could result in decreased androgen responsiveness and increased conversion of T to estrogen and its sensitivity, which are all detrimental to germ cell survival. The delayed establishment of spermatogenic functions in the Px animals is related with the delayed normalisation of serum thyroid hormone levels and the maturation of Sertoli cells.

The morphometric data of the accessory glands i.e., epididymis, seminal vesicle and prostate show that while, epididymis and prostate had a retarded growth, the seminal vesicle depicted a normal growth, though the weight of the gland was greater at all periods studied and more prominent by at 90 days. On the other hand, the epididymis and prostate showed marginal growth between 35 and 60 days and weighed significantly less than the controls. But between

60 and 90 days there was maximal growth spurt. The ultimate weight of all the three structures at 90 days was significantly greater than the controls. The histological profile also supports the morphometric changes. The measurements of epididymal tubular diameter and cell heights reveal that while the diameter was more or less identical to that of controls at all time periods, the cell height was greater at 35 and 45 days and lesser at 60 and 90 days. Taking these criteria into consideration, the significantly increased epididymal weight of the Px animals at 90 days seems to be mainly due to a greater increase in the length of the tubules. A stereological study on postnatal differentiation on the rat epididymis has demonstrated increase in the diameter of tubules and lumen, cell height and length of the tubule throughout postnatal development up to 90 days (Jiang *et al.*, 1994). The increased weight of the testes in Px animals at 90 days was attributed to an increased Sertoli and germ cell numbers. Concomitantly, as the tubular diameter was found to be slightly lesser than the controls, increase in tubular length was presumed to contribute to the testicular hypertrophy, as was reported for FSH induced testicular hypertrophy (Meachem *et al.*, 1996). Apparently, the increased epididymal weight in the Px animals at 90 days also seems to be due to increased tubular length as there was no increase in the tubular diameter or cell height. Both the morphometric and histological observations showed that the growth of the prostate is maximally retarded in the Px animals as, at 60 days, the prostate of Px animals weighed only 18% of the controls. However, at 90 days, the prostate of Px animals was heavier and histologically well differentiated. In contrast, the seminal vesicle of Px rats weighed heavier as well as showed better structural organization at all time periods compared to the controls.

The observed changes in the male sex accessory organs suggest the immediate post-pubertal period from 40-45 days onwards up to 60 days to be the period of maximum growth in control animals. However, in the Px animals, there is a delay resulting in maximum growth occurring between 60 and 90 days. These differences between control and Px animals can be related to the hormonal profile in these animals. The definite increase in T_4, T_3, PRL and T in

the period between 35-45 days in the control animals seem to exert a cumulative synergistic action resulting in the marked growth of the sex accessory organs between 45 and 60 days. However, in the Px rats though the T level was elevated the T_4, T_3 and PRL levels remain sub-normal till 45 days and tended to attain normal levels thereafter. Though T has been considered the principle hormone controlling sex accessory organ growth and maturation, recent studies with prostate have shown that T alone cannot bring about full growth and maturation, and it requires prior or synergistic action with PRL (Kharroubi and Slaunwhite, 1984). It was also shown that some of the secretory proteins of the prostatic epithelium are regulated with PRL while, some others by T (Costello and Franklin, 1994; Reiter *et al*. 1996). Even in the previous study on neonatal hypothyroidism, the delayed growth of the accessory organs was attributed to the reduced thyroid, pituitary and gonadal hormones (chapter 1). In that study, a priming action of thyroid hormones in the immature stages and synergistic action of PRL & T in the pubertal period were inferred to be the paradigms favourable for normal growth. In the present study, though the pinealectomised rats had higher T levels at 35 and 45 days, the prostatic and epididymal growth was greatly retarded, which can be attributed to the reduced thyroid hormones and PRL levels. This provides further evidence for the priming action of thyroid hormones as suggested earlier, as well as, the need for the synergistic presence for PRL. With delayed appearance of these hormones, the sex accessory organs seem to respond in a hypersensitive manner as seen in the present study. The seminal vesicle in this context appears to be least dependent on T_4/T_3 and PRL and, probably more depended on T. This is well evident in the present study as the seminal vesicle showed a very high growth rate in the Px animals right from 45 days as compared to the controls.

It can be concluded from the present study that neonatal Px increases serum gonadotropins and T and lowers PRL and thyroid hormone levels in the prepubertal period. The higher serum FSH level in the prepubertal period induces greater Sertoli cell proliferation resulting in increased germ cell number and hypertrophied heavier testes in the adult stage. The

reduced thyroxine level in the prepubertal period attenuates the overall testicular hypertrophy by delaying Sertoli cell maturation and causing attendant germ cell degeneration. Neonatal Px also causes delayed hyperplastic response of the accessory sex glands due to the reduced thyroxine and PRL levels in the early stages, thus emphasizing the need for a synergistic action of these hormones with T for proper postnatal growth and maturation of the male sex accessory organs.