# CHAPTER 1

## TRANSIENT NEONATAL HYPOTHYROIDISM DECREASES ADULT TESTES SIZE AND SEX ACCESSORY ORGAN WEIGHTS IN THE RAT: POSSIBLE STRAIN DIFFERENCE

Thyroid hormone is essential for overall growth and development of mammals and, its importance in the fetal and post-natal periods has been elucidated. The importance of thyroid hormones in the pre-natal and post-natal periods becomes obvious from the reported increase in plasma concentrations of tri-iodothyronine ($T_3$ ) and thyroxine ($T_4$) by six and four fold respectively between the end of gestation and weaning in the rat (Canavan *et al.*, 1994). There have been many attempts to relate thyroid hormones with development and functions of the male reproductive system. Some of the early studies showed that the growth of the testis and epididymis is only slightly retarded in hypothyroid (HPOT) rats (Hammet, 1923; Del Rio *et al.*, 1979), but some later reports showed that thyroidectomy in immature male rats can cause severe inhibition of spermatogenesis and Leydig cell development (Chowdhury *et al* ., 1984; Weiss and Burns, 1988). Thyroidectomy or goitrogen treatment was reportedly ineffective on the functions of testis in the adult rat (Vilchez-Martinez, 1973; Amin and El-Sheikh, 1977). However, Baksi (1973) showed degenerative changes in the testis due to hypothyroidism. Recently, differential effects on testicular development have been shown by different schedules of goitrogen treatment. Accordingly chronic hypothyroidism induced by methimazole administration

from birth to puberty caused a delay in the maturation of the seminiferous tubules and reduced their diameter and the number of germ cells and arrested their maturation, leading to reduced final testis size (Chowdhury and Arora, 1984; Chowdhury *et al.*, 1984,Valle *et al.*, 1985; Francavilla *et al.*, 1991; Meisami *et al.*, 1992; Van Haaster *et al.*, 1993). In contrast, one month old pre-pubertal rats when thyroidectomised (Tx) or, on goitrogen administration in post-pubertal rats, were without any effect on testis growth and fertility (Vilchez-Martinez, 1973; Kurez *et al* ., 1974; Kalland *et al.*, 1978; Weiss and Burns, 1988; Maya *et al.*, 1990).

However, induction of transient hypothyroidism during the first three weeks after birth by the administration of the reversible goitrogen propyl-thiouracil (PTU) has been shown to increase, adult testis and reproductive organ size (Cooke, 1991; Cooke and Meisami, 1991; Cooke *et al.*, 1992; 1993; 1994a; 1994b; Kirby *et al.*, 1992; Meisami *et al.*, 1992; Van Haaster, 1992; Meisami *et al.*, 1994), sperm production (Cooke *et al.*, 1991; Kirby *et al.*, 1992), and Sertoli and germ cell numbers (Hess *et al.*, 1993; Simorangkir *et al.*, 1995). These reports are based on studies conducted on Long-Evans and Sprague-Dawley strains of rats. It is likely that different strains of rats might respond differentially due to their inherent genetic differences. In this respect, the present study deals with the evaluation of the effects on the functional maturation and the development of the male reproductive system and that of thyroid in Charles-foster strain of rats subjected to transient neonatal hypothyroidism.

## RESULTS

### I. MORPHOMETRIC OBSERVATIONS

BODY WEIGHT (Table 1.1; Fig. 1 & 2)

The body weight was greater in the control animals at 35 days than that of the hypothyroid animals. The control animals showed continuous increase in body weight from 35 to 90 days except for a slight decline between 35 and 45 days. The hypothyroid (HPOT) animals showed only marginal increment in body weight between 35 and 60 days, however, the body weight

Table 1.1 Chronological alterations in Body Weight (gm), Percentage Difference and Per Day Growth Rate in intact and hypothyroid (HPOT) 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 |
| HPOT | 19.76 ± 1.43$^{d}$ | 24.50 ± 2.87$^{d}$ | 32.50 ± 2.42$^{d}$ | 120.13 ± 4.56$^{ns}$ | + 23.99 | + 32.65 | + 269.63 | + 507.95 | 0.56 ± 0.02$^{d}$ | 0.47 ± 0.01$^{d}$ | 0.53 ± 0.01$^{d}$ | 2.90 ± 0.15$^{d}$ |

@ Values expressed as Mean ± SD of five experiments

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

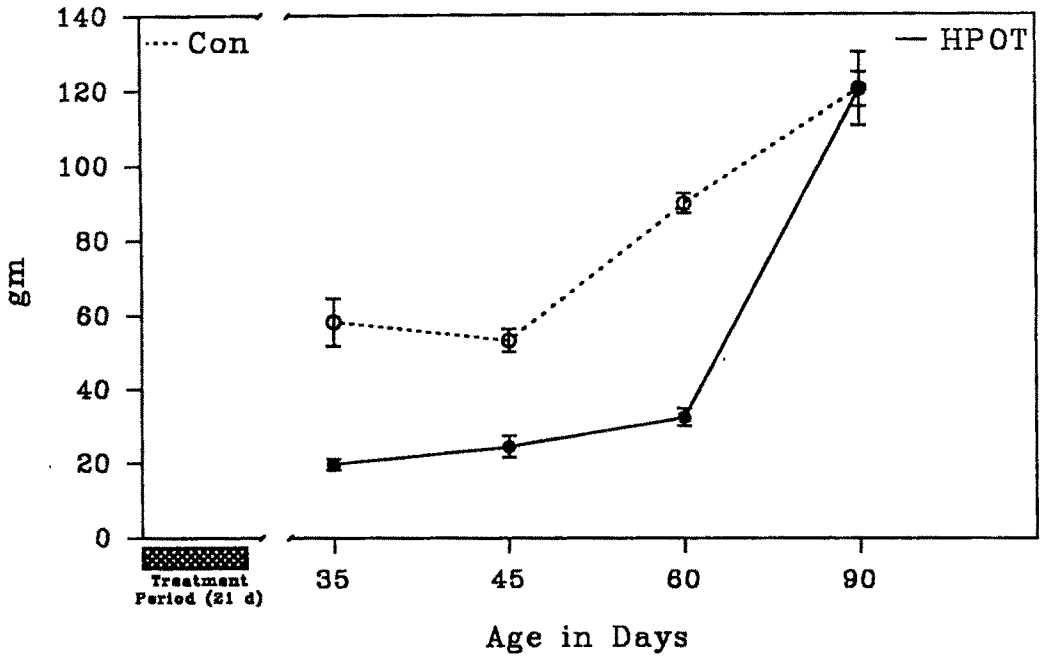



Fig. 1 Chronological alterations in body weight of neonatal rats subjected to transient hypothyroidism (HPOT)

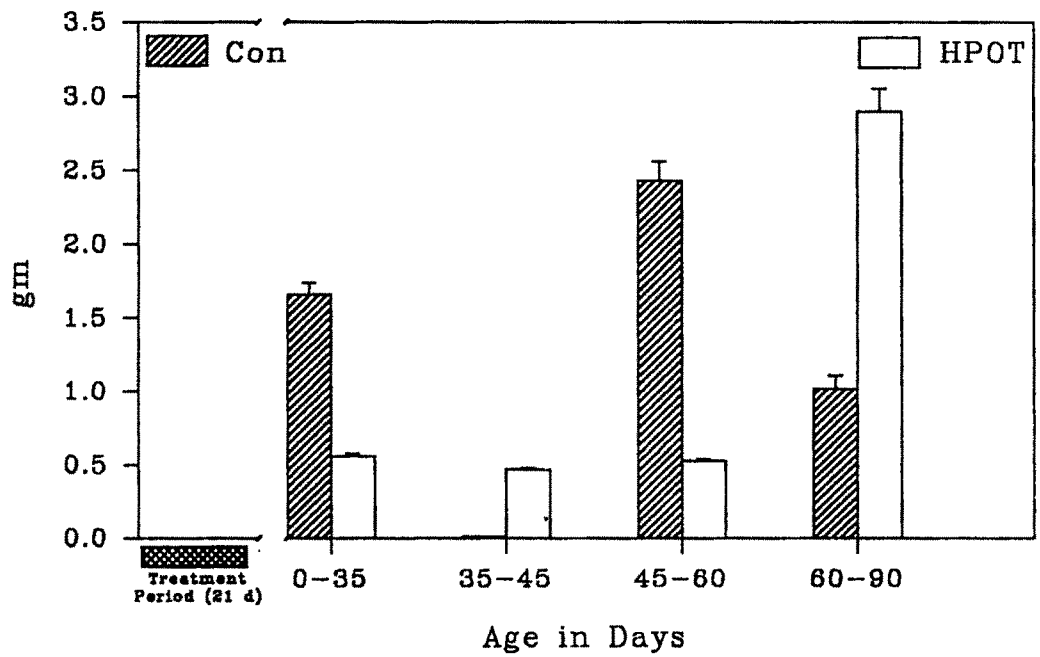



Fig. 2 Per day body growth in intact and hypothyroid rats

increased very significantly between 60 and 90 days. Both the control and HPOT animals had similar body weight at 90 days. On a percentage basis, the increase in body weight between 35 and 90 days was greater in HPOT animals as compared to the controls.

ORGAN WEIGHTS

**Testes** (Table 1.2 a, b; Fig. 3 a, b & 4)
The HPOT rats had significantly low testes weight at 35 days than that of the control. The testes weight increased continuously and steadily between 35 and 90 days in control animals. In the HPOT animals, the testes weight increased very significantly between 60 and 90 days with a constant weight between 45 and 60 days. The final testes weight at 90 days was significantly less in HPOT compared to control rats. In terms of relative weight also, the HPOT animals had lesser testes weight at 35 days. The relative weight of testes showed continuous increase in control animals between 35 and 90 days. However, in the HPOT rats, the relative weight increased between 35 and 45 days and, again between 60 and 90 days with a decrease between 45 and 60 days.

**Epididymis** (Table 1.2 a, b; Fig. 5 a, b & 6)
The epididymis in HPOT animals weighed significantly lesser than the controls. In the control animals, the weight of the epididymis decreased significantly at 45 days compared to 35 days, while in HPOT animals, there was a marginal but less significant increase. Between 45 and 90 days the weight of the epididymis increased continuously and significantly in control animals. Whereas the maximal growth spurt was between 45 and 60 days in control animals, the HPOT animals showed very little increase in weight upto 60 days but, between 60 and 90 days there was a very pronounced growth spurt. The ultimate absolute weight of epididymis at 90 days was significantly less in HPOT rats compared to controls. Relative weight of epididymis in both control and HPOT animals paralleled the changes in absolute weight at all stages.

Table 1.2 (a & b)Chronological alterations in Weight [Absolute (mg) and Relative (mg/100 mg)], Percentage Difference and Per Day Growth Rate of Testes and Epididymis in intact (Con) and hypothyroid (HPOT) 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 |
| | HPOT | 80.26$^{d}$ ± 3.94 | 149.32$^{d}$ ± 10.29 | 144.88$^{d}$ ± 9.93 | 1714.57$^{d}$ ± 39.22 | 0.41$^{d}$ ± 0.02 | 0.62$^{d}$ ± 0.10 | 0.45$^{d}$ ± 0.04 | 1.43$^{d}$ ± 0.03 |
| EPIDIDYMIS | Con | 122.86 ± 8.37 | 64.28 ± 2.27 | 394.40 ± 10.24 | 503.84 ± 15.43 | 0.22 ± 0.04 | 0.12 ± 0.01 | 0.44 ± 0.02 | 0.42 ± 0.02 |
| | HPOT | 31.70$^{d}$ ± 3.38 | 40.76$^{d}$ ± 6.17 | 44.7$^{d}$ ± 4.19 | 352.18$^{d}$ ± 9.94 | 0.12$^{c}$ ± 0.02 | 0.12$^{d}$ ± 0.01 | 0.14$^{d}$ ± 0.01 | 0.29$^{d}$ ± 0.003 |

Table b

| ORGAN | TREATMENT | PERCENTAGE DIFFERENCE | | | | PER DAY GROWTH RATE | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Age in Days | | | | Age in Days | | | |
| | | 35-45 | 45-60 | 60-90 | 35-90 | 0-35 | 35-45 | 45-60 | 60-90 |
| TESTES | Con | + 52.46 | + 92.06 | + 74.53 | + 411.06 | 13.63 ± 2.32 | 25.03 ± 3.42 | 44.60 ± 4.05 | 34.70 ± 4.95 |
| | HPOT | + 86.04 | - 2.97 | + 1083.44 | + 2036.27 | 2.29$^{d}$ ± 0.12 | 6.90$^{d}$ ± 0.99 | - | 52.32$^{d}$ ± 6.56 |
| EPIDIDYMIS | Con | - 47.68 | + 513.56 | + 27.75 | + 310.09 | 3.51 ± 0.32 | - | 22.06 ± 6.45 | 3.65 ± 0.24 |
| | HPOT | + 28.58 | + 9 67 | + 687.87 | + 1010 98 | 0.91$^{d}$ ± 0.02 | 0.91$^{d}$ ± 0.03 | 0.26$^{d}$ ± 0.009 | 10.25$^{d}$ ± 2.65 |

@ Values expressed as Mean ± SD of five experiments; $^{c}$ $p < 0.01$; $^{d}$ $p < 0.001$

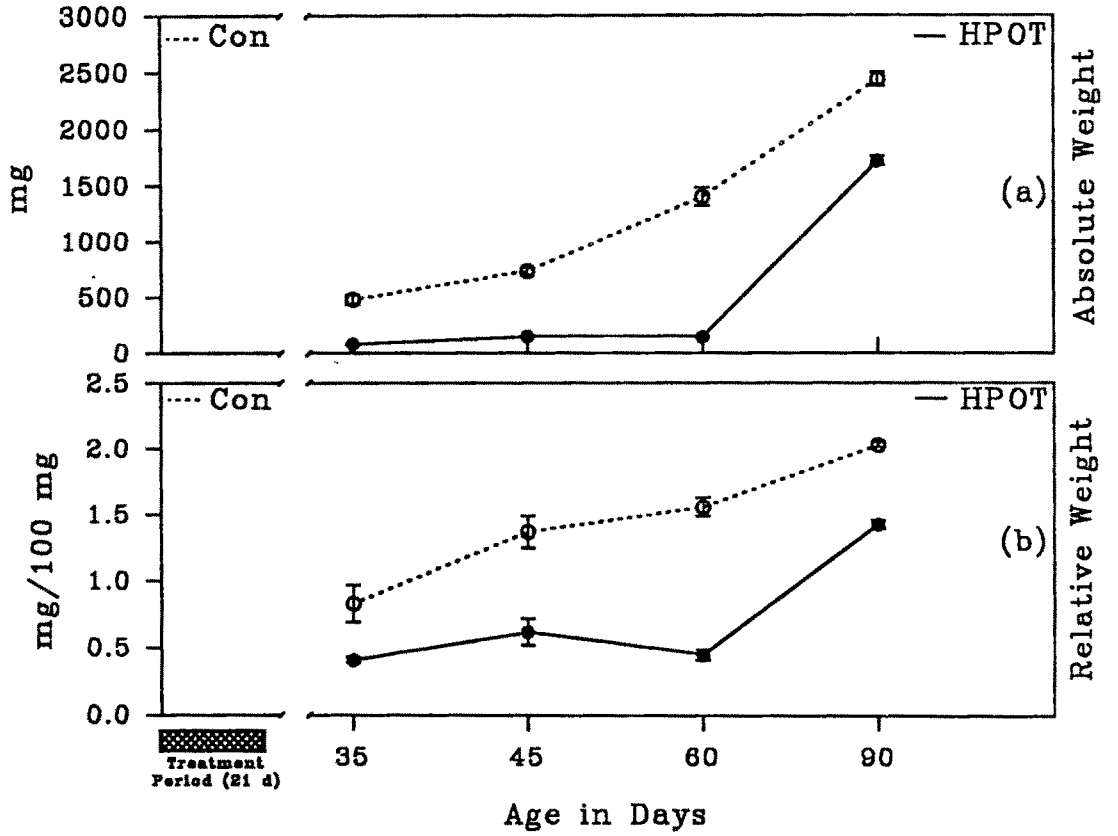



Fig. 3 (a&b) Chronological alterations in absolute and relative weights of testes in intact and hypothyroid (HPOT) rats

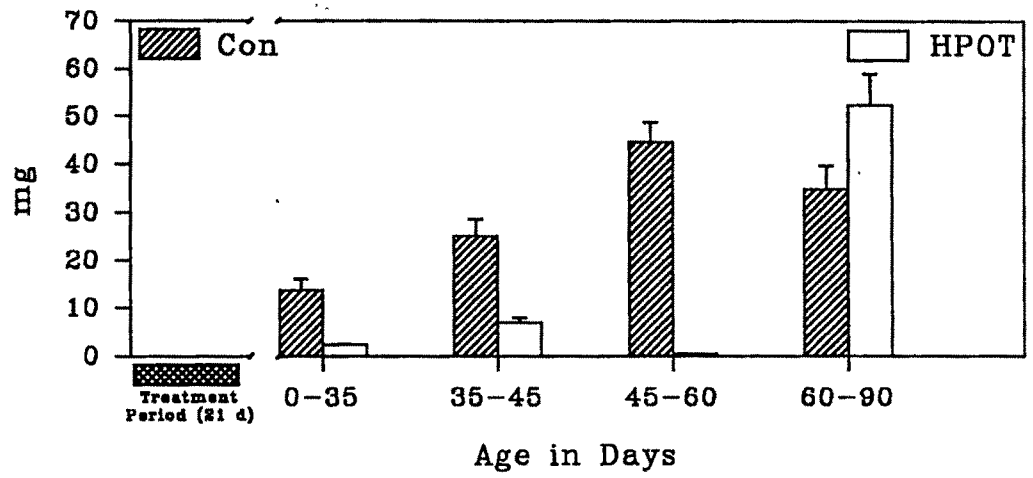



Fig. 4 Rate of growth of testes in intact and hypothyroid rats

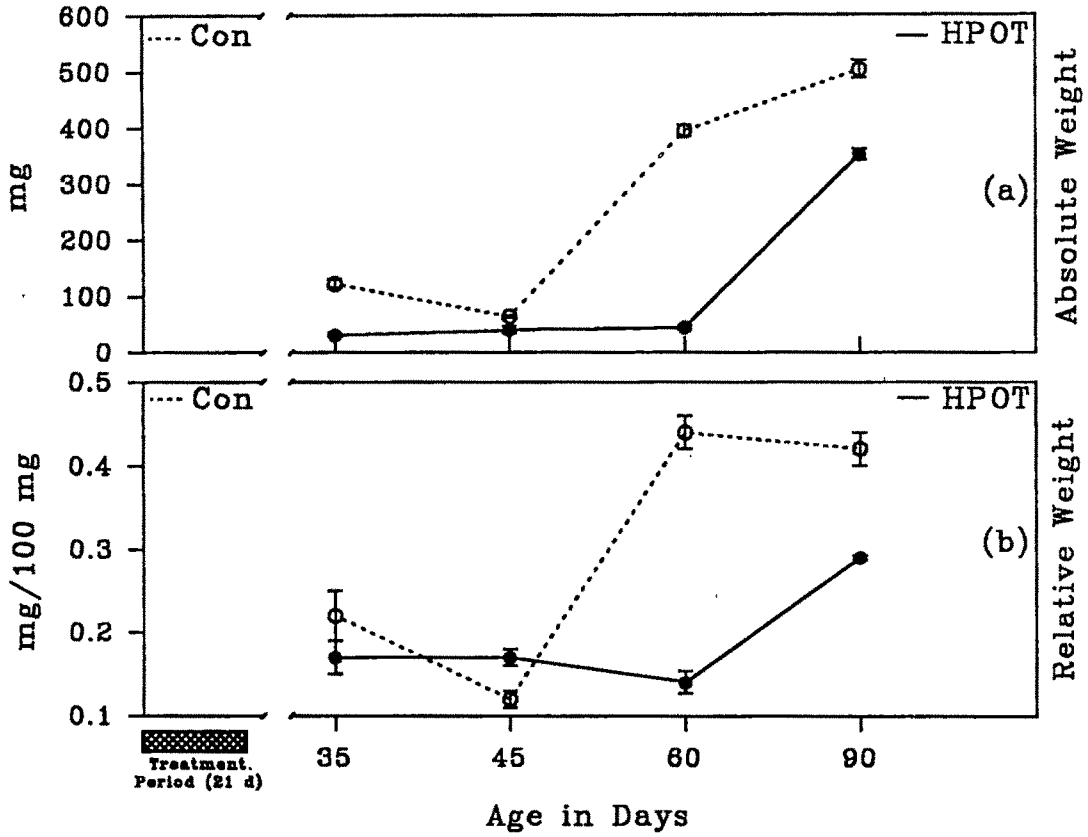



Fig. 5 (a&b) Chronological alterations in absolute and relative weights of epididymis in intact and hypothyroid (HPOT) rats

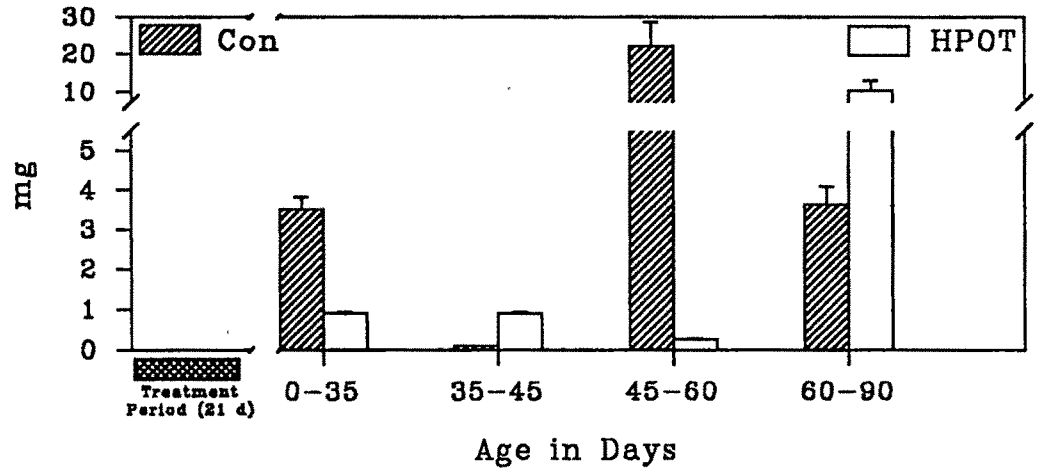



Fig. 6 Rate of growth of epididymis in intact and hypothyroid rats

**Seminal Vesicle** (Table 1.3 a, b: Fig. 7 a, b & 8)

At 35 days, the absolute weight of seminal vesicle was significantly lesser in HPOT animals compared to controls. The weight of the seminal vesicle showed a decrease at 45 days in the control animals, while in the HPOT animals there was a marginal increase. Between 45 and 90 days the weight of seminal vesicle increased continuously and significantly in the control animals with the maximum growth spurt occurring between 60 and 90 days. The HPOT animals showed no change in weight between 45 and 60 days but then showed a tremendous increase between 60 and 90 days. The final weight of the seminal vesicle at 90 days was more in control than the HPOT animals. The changes in the relative weights of seminal vesicles in both the groups at all the stages reflected the changes in the absolute weight.

**Prostate Gland** (Table 1.3 a, b: Fig. 9 a, b & 10)

The weight of prostate gland was significantly high in controls at 35 days than that of the HPOT animals. There was a decrement in weight at 45 days in the control animals. The HPOT animals showed less pronounced but significant increase. In control animals, the weight of prostate gland increased continuously between 45 and 90 days with a maximal growth rate between 45 and 60 days, while in the HPOT animals, there was a non-significant increase in the weight of prostate gland between 45 and 60 days, followed by a tremendous increase between 60 and 90 days. The ultimate weight of the prostate gland in control rats at 90 days was more than the HPOT rats. The relative weight of prostate gland in both control and HPOT animals at all the stages reflected the changes in absolute weight.

**Thyroid Gland** (Table 1.4; Fig. 11 a & b)

The weight of the thyroid gland was significantly high in HPOT animals at 35 days than the control animals. Both groups of animals depicted a significant decrement in the weight of thyroid at 45 days, more pronounced in the control animals. Between 45 and 90 days, the thyroid weight increased in both control and HPOT rats. Ultimately, the weight of the thyroid was significantly greater in HPOT animals at 90 days. Whereas the maximal weight gain in thyroid gland in control

Table 1.3 (a & b)Chronological alterations in Weight [Absolute (mg) and Relative (mg/100 mg)], Percentage Difference and Per Day Growth Rate of Seminal Vesicle and Prostate Gland in intact (Con) and hypothyroid (HPOT) 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 |
| | HPOT | 6.08$^{d}$ ± 0.75 | 8.90$^{d}$ ± 1.02 | 8.05$^{d}$ ± 0.90 | 311.82$^{d}$ ± 12.21 | 0 031$^{c}$ ± 0.004 | 0.037$^{ns}$ ± 0.005 | 0.025$^{d}$ ± 0.002 | 0.259$^{d}$ ± 0.002 |
| 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 |
| | HPOT | 8.66$^{d}$ ± 0.65 | 13.47$^{ns}$ ± 1.78 | 15.25$^{d}$ ± 1.92 | 120.75$^{c}$ ± 10.71 | 0.043$^{ns}$ ± 0.003 | 0.055$^{d}$ ± 0.005 | 0.047$^{d}$ ± 0.003 | 0.10$^{d}$ ± 0.002 |

Table b

| ORGAN | TREATMENT | PERCENTAGE DIFFERENCE | | | | PER DAY GROWTH RATE | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Age in Days | | | | Age in Days | | | |
| | | 35-45 | 45-60 | 60-90 | 35-90 | 0-35 | 35-45 | 45-60 | 60-90 |
| SEMINAL VESICLE | Con | - 24.21 | + 252.32 | + 533.06 | + 1590.35 | 0.72 ± 0.06 | - | 3.19 ± 0.28 | 11.89 ± 1.05 |
| | HPOT | + 47.69 | - 9.55 | + 3773.54 | + 5028.62 | 0.17$^{d}$ ± 0.02 | 0.28$^{d}$ ± 0.02 | - - | 10.13$^{ns}$ ± 0.99 |
| PROSTATE GLAND | Con | - 37.94 | + 435.51 | + 89.67 | + 531.03 | 0.69 ± 0.09 | - | 4.41 ± 0.95 | 2.43 ± 0.89 |
| | HPOT | + 55.54 | + 13.21 | + 691.61 | + 1293.99 | 0.25$^{d}$ ± 0.01 | 0.48$^{d}$ ± 0.01 | 0.12$^{d}$ ± 0.009 | 3.51$^{c}$ ± 0.18 |

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

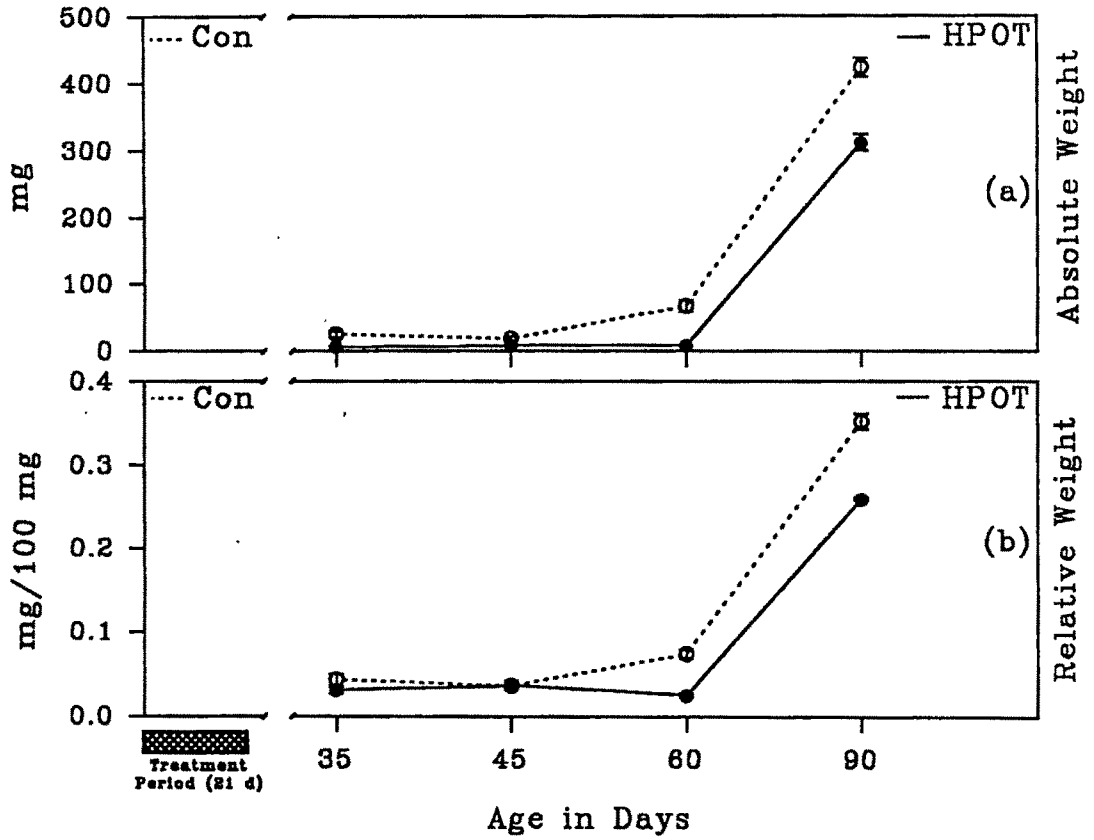



Fig. 7 (a&b) Chronological alterations in absolute and relative weights of seminal vesicle in intact and hypothyroid (HPOT) rats

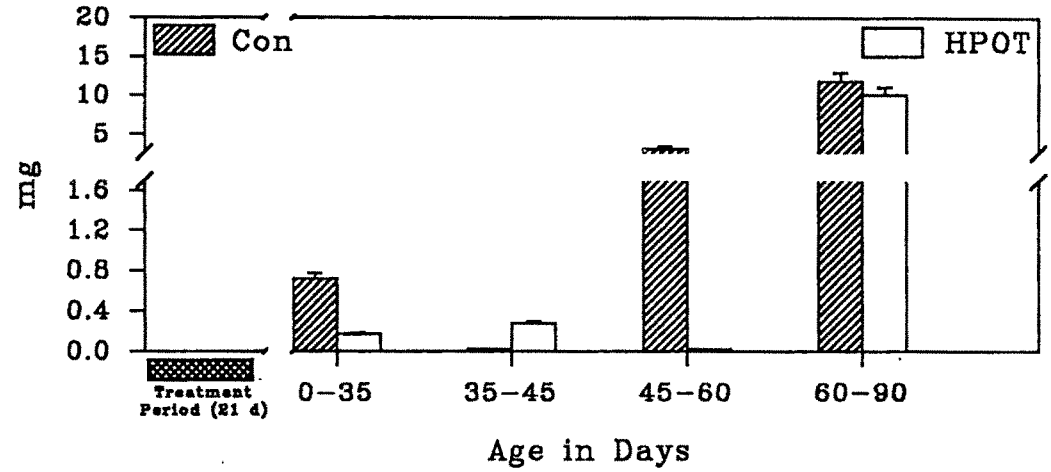



Fig. 8 Rate of growth of seminal vesicle in intact and HPOT rats

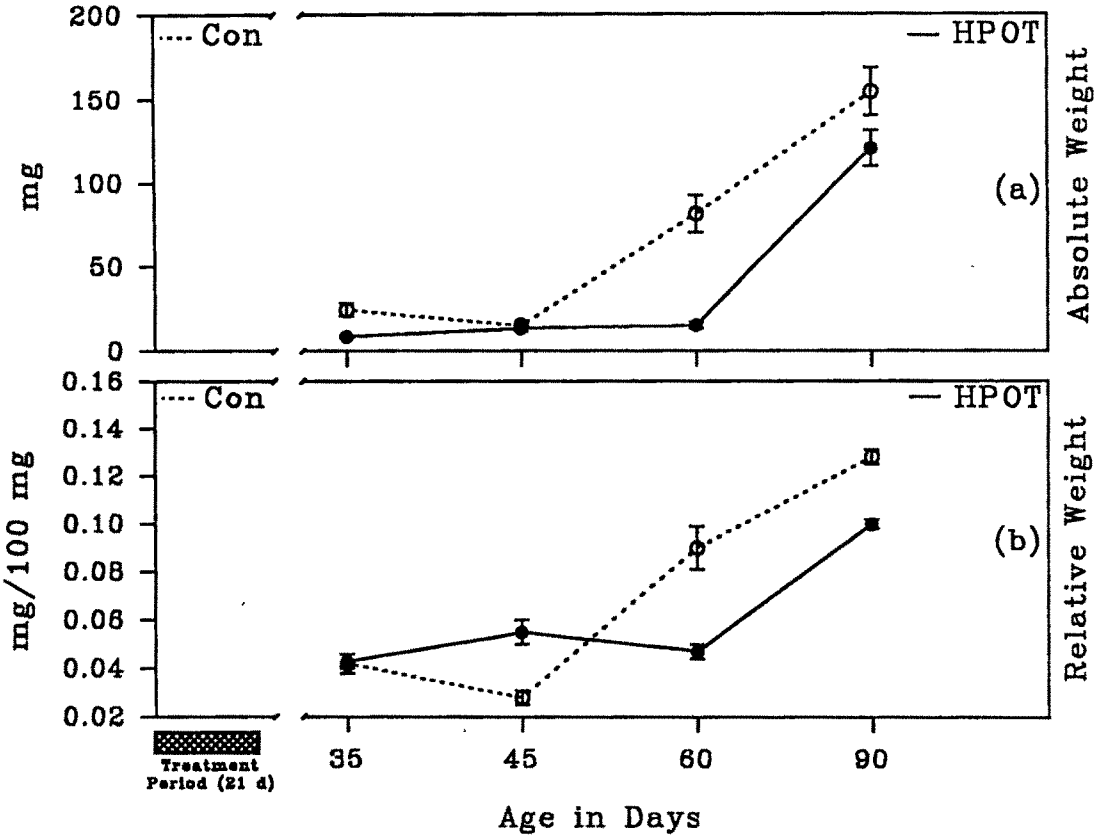



Fig. 9 (a&b) Chronological alterations in absolute and relative weights of prostate gland in intact and hypothyroid (HPOT) rats

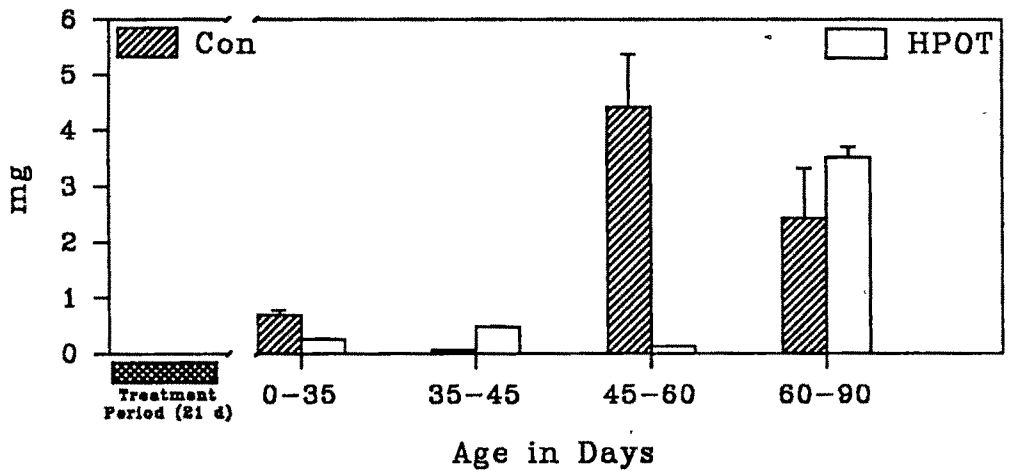



Fig. 10 Rate of growth of prostate gland in intact and HPOT rats

Table 1.4 Chronological alterations in Weight [Absolute (mg) and Relative (mg/100mg)] and Percentage Difference of Thyroid Gland in intact and hypothyroid (HPOT) 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 |
| HPOT | 10.56 ± 1.37$^{d}$ | 7.52 ± 0.50$^{d}$ | 6.56 ± 0.50$^{ns}$ | 11.78 ± 1.52$^{d}$ | 0.053 ± 0.005$^{d}$ | 0.031 ± 0.005$^{d}$ | 0.02 ± 0.001$^{d}$ | 0.009 ± 0.0004$^{ns}$ | + 28.79 | - 12.76 | + 79.57 | + 11.36 |

@ Values expressed as Mean ± SD of five experiments

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

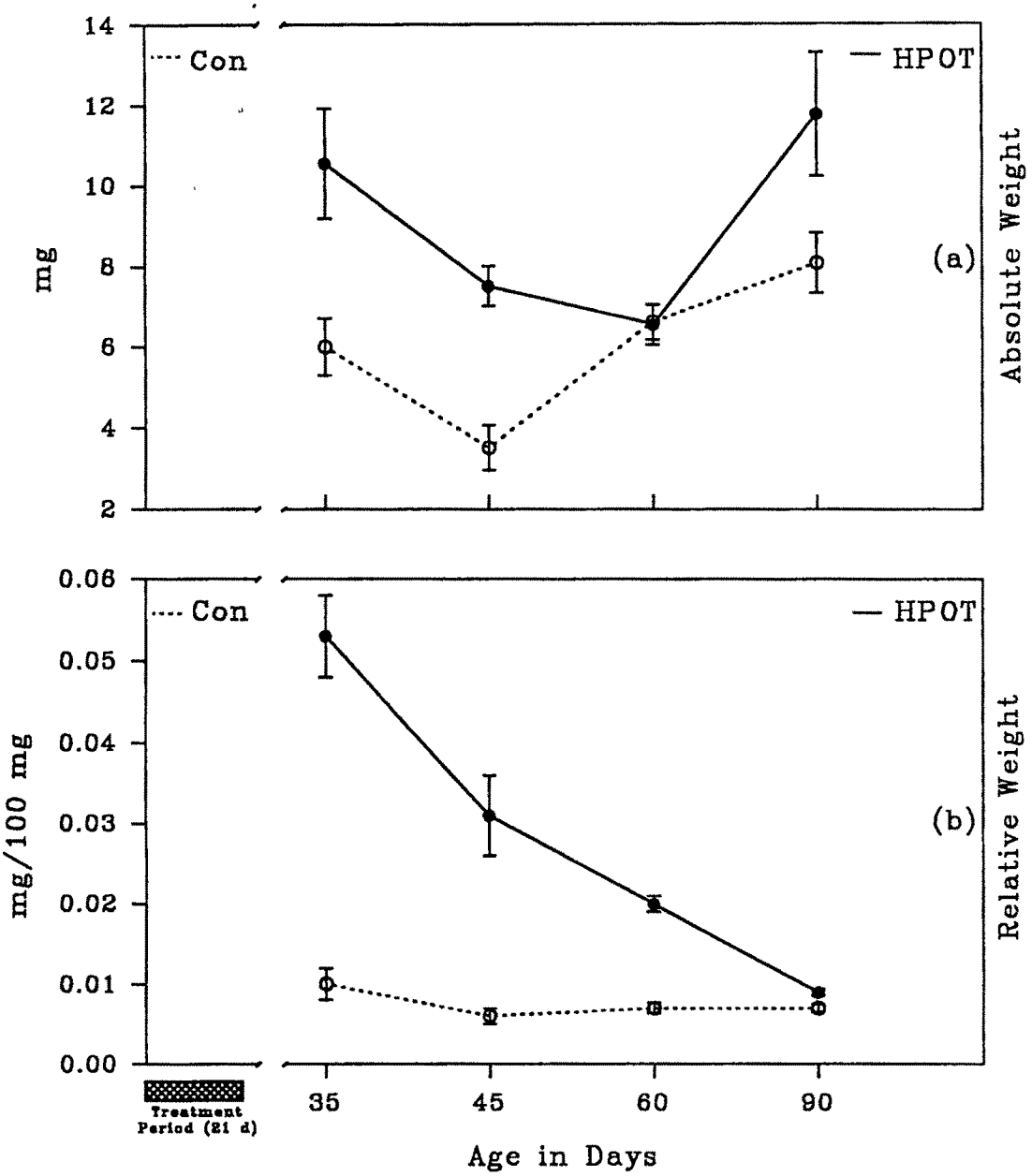



Fig.11 (a&b) Chronological alterations in absolute and relative weights of thyroid gland in intact and hypothyroid (HPOT) rats

animals occurred between 45 and 60 days, the same occurred in HPOT animals between 60 and 90 days. The relative weight of thyroid gland was maximal at 35 days in the HPOT rats. The relative weight of thyroid gland at 90 days was minimal for both the groups, though the HPOT had greater relative weight than the control groups of rats.

## II. HISTOLOGICAL OBSERVATIONS

STRUCTURE OF TESTIS (Table 1.5; Plates I-III)

**35 Day Old**

Control: The tubules were small with an average diameter 90.47 $\mu$m with mostly spermatogonial cells and primary spermatocytes in the zygotene stage. Some tubules also showed few pachytene spermatocytes. The tubules were lumenated 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 could be seen.

HPOT: The tubules were smaller than controls with an average diameter of 76.19 $\mu$m. The overall germ cell population appeared to be quantitatively less. Many of the primary spermatocytes showed degenerative changes with pyknotic nuclei. The spermatogonial compartment also appeared to have lesser number of cells. The interstitial cells were mostly fibroblast like though, in some areas, there were some prominent cells.

**45 Day Old**

Control: The tubules were enlarged with an average diameter of 114.28 $\mu$m. 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.

HPOT: The tubules increased in size slightly, with an average diameter of 88.57 $\mu$m, similar to the diameter of the tubules at 35 days in the control animals. Germ cell degeneration which was

Table 1.5 Chronological alterations in the Diameter (in $\mu$m) of Seminiferous Tubule and Epididymis (Caput and Cauda) in intact and hypothyroid (HPOT) 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 |
| HPOT | 76.19 ± 4.14$^{c}$ | 88.57 ± 8.46$^{d}$ | 103.90 ± 9.03$^{d}$ | 137.91 ± 11.99$^{d}$ | 29.57 ± 3.21$^{d}$ | 26.91 ± 2.48$^{ns}$ | 23.95 ± 2.95$^{d}$ | 32.24 ± 2.68$^{d}$ | 17.05 ± 1.27$^{d}$ | 17.21 ± 1.17$^{c}$ | 16.67 ± 1.29$^{b}$ | 16.19 ± 1.21$^{b}$ |

@ Values expressed as Mean ± SD of five experiments

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

## PLATE I

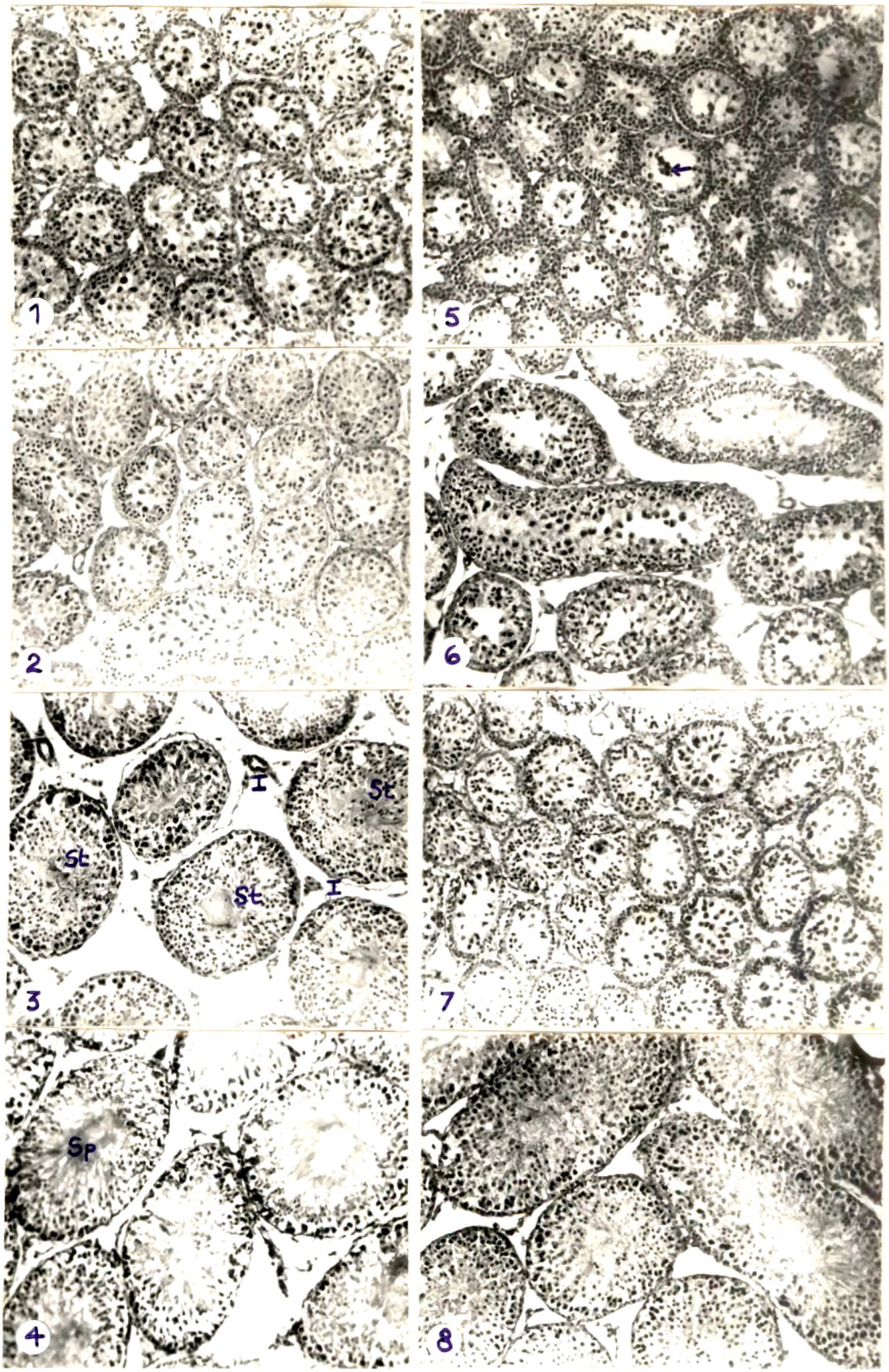
Photomicrographs of sections of testis of control and hypothyroid (HPOT) rats showing histological features at 35, 45, 60 and 90 days (100 x).

Figures 1 and 5: Sections of testis of 35 day old control and HPOT rats respectively showing the presence of more germ cells and spermatocytes up to pachytene stage in the former and the reduced number of germ cells, with degenerating germ cells (arrow) in the latter. Note the reduced tubular diameter in the HPOT rats.

Figures 2 and 6: Sections of testis of 45 day old control and HPOT rats respectively showing progression of spermatogenesis in the former and arrest of germ cell degeneration and reestablishment of spermatogenesis in the latter.

Figures 3 and 7: Sections of testis of 60 day old control and HPOT rats respectively showing larger tubules with the appearance of spermatids (St) in the former and progression of spermatogenesis into the post meiotic stages impeded in the latter. Note well formed interstitium (I) in the controls.

Figures 4 and 8: Sections of testis of 90 day old control and HPOT rats showing appearance of sperms (Sz) in the former and establishment of spermiogenesis in the latter.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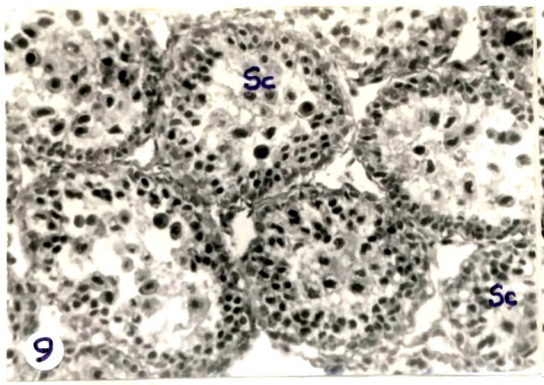
## PLATE II

Photomicrographs of sections of testis of control and HPOT rats showing histological features at 35, 45, and 60 days (200 x).

Figures 9 and 12: Enlarged versions of 35 day old control and HPOT testis shown in figures 1 and 5. Note the presence of spermatocytes (Sc) in the control (Fig. 9) and their degeneration (arrow) in the HPOT rats (Fig. 12).

Figures 10 and 13: Enlarged versions of 45 day old control and HPOT rat testis. Note the appearance of round spermatids (RSt) in the control (Fig. 10) and establishment of spermatogenesis marked by the appearance of the spermatocytes (Sc) in the HPOT (Fig. 13)

Figures 11 and 14: Enlarged photomicrographs of sections of testis of 60 day old control and HPOT rats. Note the larger diameter of the tubules and presence of elongating spermatids (ESt) with prominent interstitium (I) in the former (Fig. 11) and the smaller tubular diameter with fewer germ cells and spermatocytes (Sc) in the latter (Fig. 14)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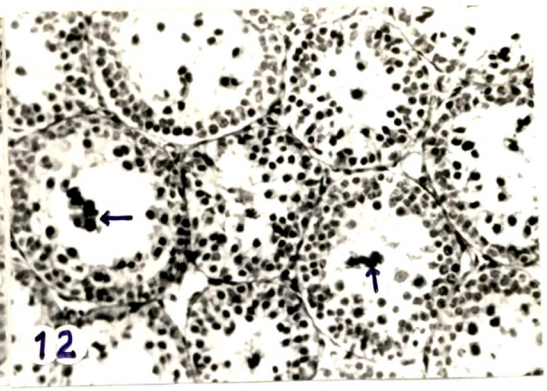
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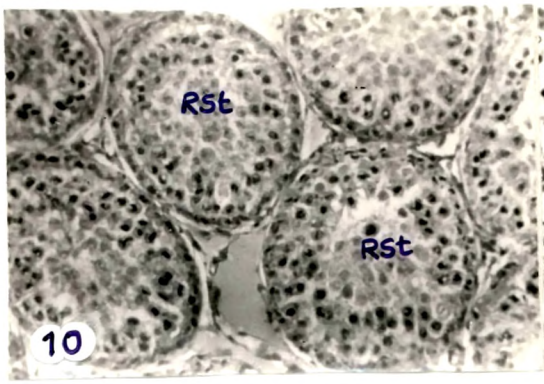
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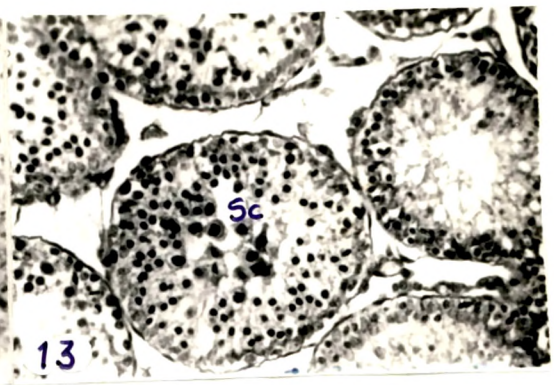
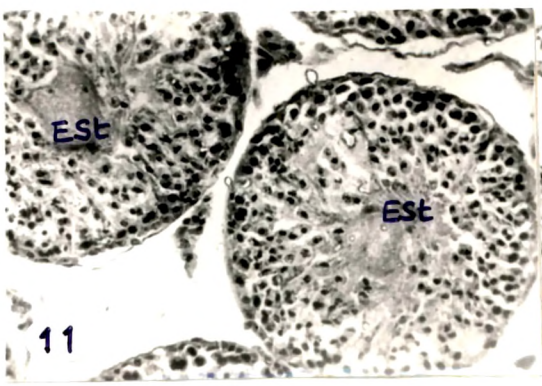
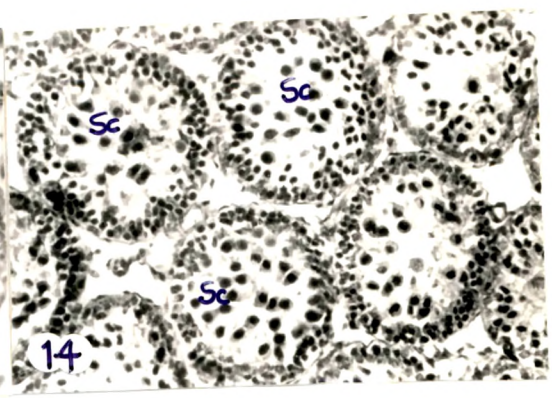

Sc
13


ESt
ESt
11

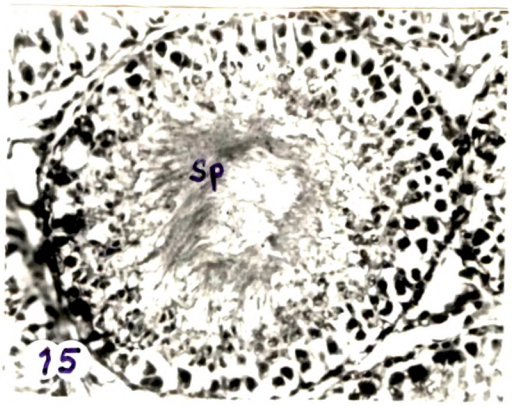

Sc
Sc
Sc
14

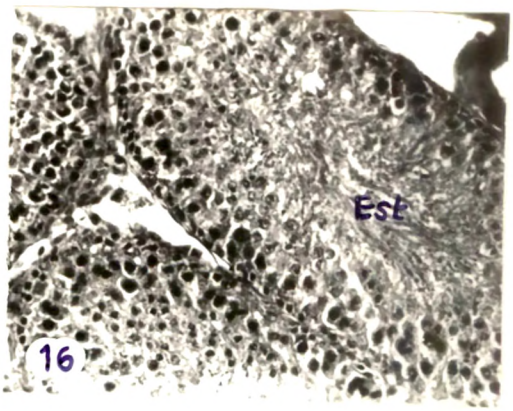
## PLATE III

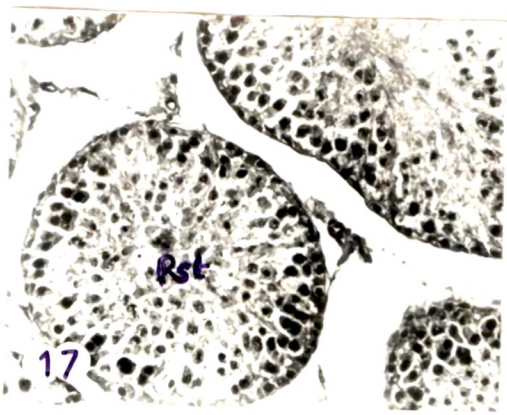
Figures 15 to 17- Photomicrographs of sections of testis of control and HPOT rats (200x)

Figure 15: Section of control testis showing a single tubule showing the presence of spermatozoa (Sp).

Figures 16 and 17: Sections of testis of HPOT rats showing the presence of only round spermatids(RSt) in some tubules (Fig. 17) and elongating spermatids (ESt) in some (Fig. 16).


Sp
15


Est
16


Rst
17

evident at 35 days seemed to be checked. Spermatogenesis appeared to be re-established with many zygotene spermatocytes. The interstitial cells showed signs of activation.

**60 Day Old**

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

HPOT: There was only a slight increase in the diameter of the tubules from 45 days and measured only 103.9 $\mu$m, which was significantly less than the controls. The tubules were compactly packed with intense spermatogonial proliferation. Spermatocyte population appeared to be lesser with many tubules showing, the meiotic cells hypertrophied and undergoing degeneration. Progression through the meiotic cell types seems to be affected. The effect appears to be differential, with some of the tubules depicting better functional integrity marked by the presence of even spermatids. Apparently, spermatogenesis seems to be reestablished in many tubules though, the spermatocyte population was quantitatively less. The interstitium was not prominent and appeared to be mostly fibroblast like.

**90 Day Old**

Control: The tubules were further enlarged with a maximum diameter of 187.52 $\mu$m. Spermatogenesis was fully established in all the tubules and, most of the tubules were having sperms. The interstitium appears to be moderately developed.

HPOT: Tubules showed distinct enlargement compared to 60 days and, measured 137.90 $\mu$m, which was less than that of control rats. There was increase in germ cell number and spermatogenesis was established. Some of the tubules showed spermatids and spermatozoa. However, many of the tubules still showed hypertrophied, degenerating germ cells. Even in those tubules where sperms were evident, their population appeared to be less. Interstitium was prominent.

STRUCTURE OF EPIDIDYMIS (Table 1.5; Plates IV & V)

**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 $\mu$m. In between the tubules, fibrous connective tissue was evident. Degenerated germ cells flushed out from the testis could be seen in the lumen.

HPOT: The tubules were loosely organised and the cells were hypertrophied with the cell height varying between 17.05 to 29.57 $\mu$m, greater than that of the controls. Degenerating germ cells could be seen in the lumen.

**45 Day Old**

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

HPOT: The tubules were large with hypertrophied cells whose diameter was about 26.9 $\mu$m. The lumen appeared relatively narrow and contained 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 $\mu$m.

HPOT: The tubules were well formed and the cell height was distinctly less than that of 45 days, ranging between 16.7 to 23.95 $\mu$m. This was evidently lesser than that of 60 day control and Px animals. The lumen appeared narrow with no germ cells.

**90 Day Old**

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

HPOT: The tubules were large lined by prominent cuboidal to columnar epithelial cells with cell height varying between 16.2 to 32.24 $\mu$m, which was less than that of the controls. The lumen

## PLATE IV

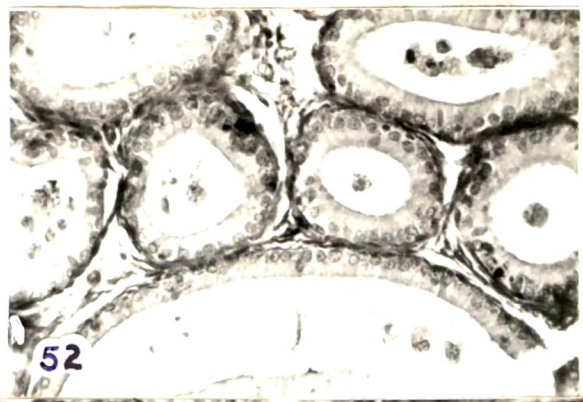
Figures 52 to 57- Photomicrographs of sections of epididymis of 35, 45, 60, and 90 day old control rats (200 x).

Figure 52: The section of epididymis of 35 day old rat showing tubules lined by cuboidal to columnar epithelium. Degenerated germ cells flushed out can be seen in the lumen.

Figures 53 and 54: Sections of epididymis of 45 day old rat showing well developed compactly packed tubules. The lumen is seen filled with round spermatids.

Figure 55: Section of epididymis of 60 day old rat showing well developed and compactly packed tubules.

Figures 56 and 57: Sections of epididymis of 90 day old rat showing large and well formed tubules lined by tall columnar epithelial cells and lumen filled with spermatozoa.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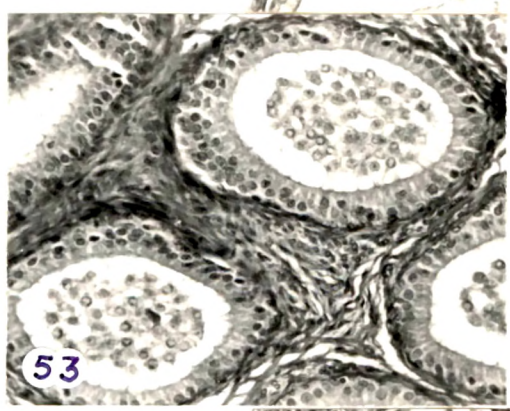
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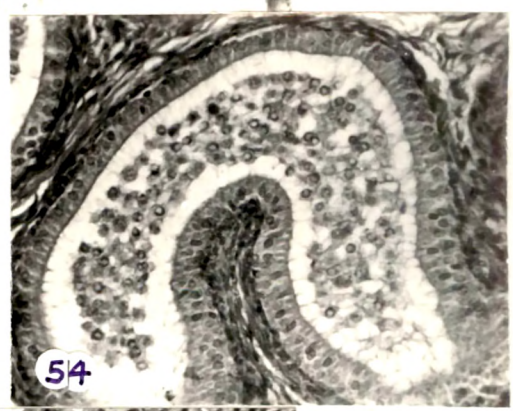
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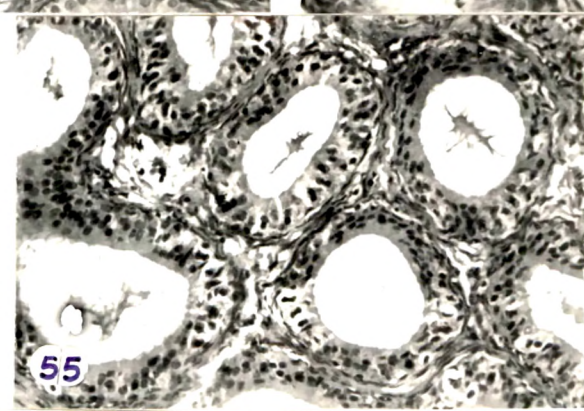
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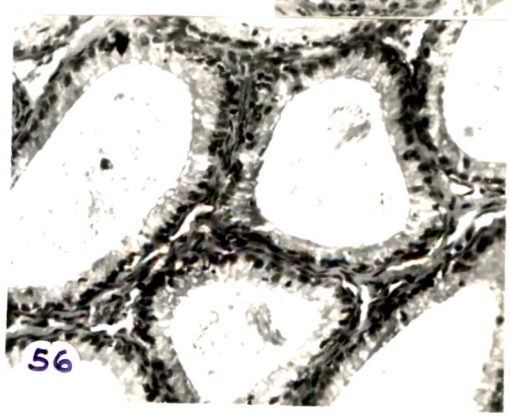
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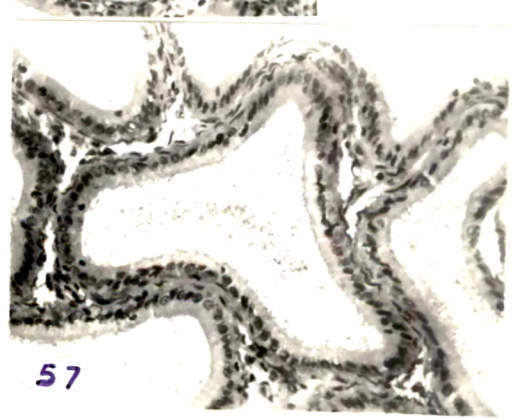

57

## PLATE V

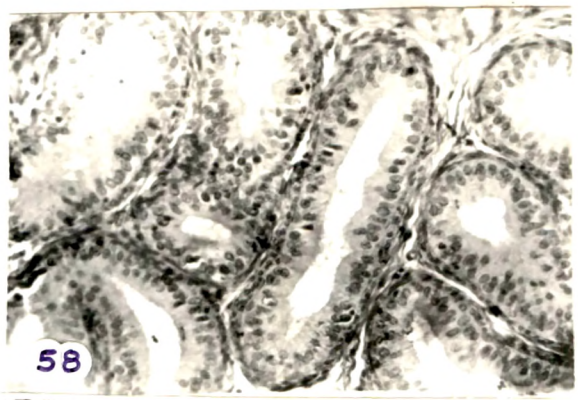
Figures 58 to 62- Photomicrographs of sections of epididymis of 35,45, 60 and 90 day old HPOT rats (200 x).

Figure 58: Section of epididymis of 35 day old rat showing loosely organised tubules with hypertrophied cells.

Figure 59: Section of epididymis of 45 day old rat showing slightly enlarged tubules lined by hypertrophied cells and the lumen containing degenerating germ cells.

Figure 60: Section of epididymis of 60 day old rat showing well formed tubules with prominent epithelial cells.

Figures 61 and 62: Sections of 90 day old rat showing tubules lined by prominent cuboidal epithelial cells. The lumen contains amorphous material with germ cells.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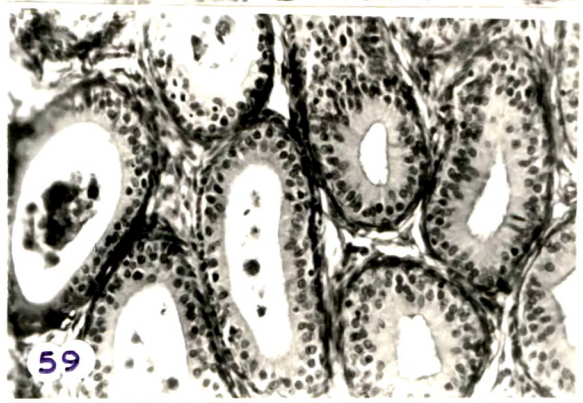
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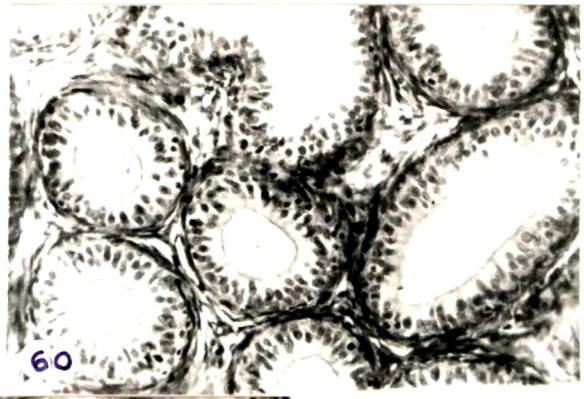
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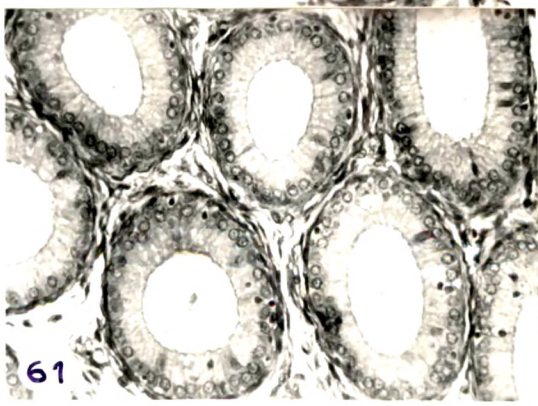
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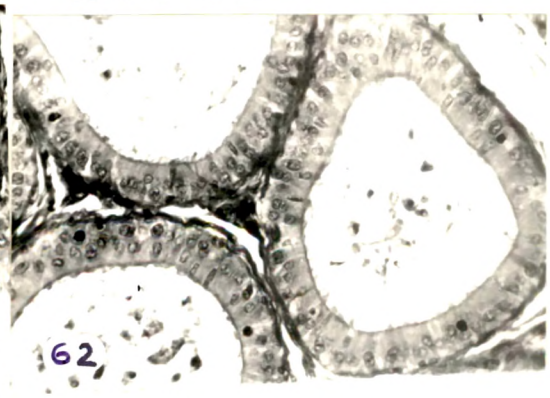

62

contained amorphous material with germ cells (obviously, the cell height remains constant from 35 days).

STRUCTURE OF SEMINAL VESICLE (Plates VI & VII)

**35 Day Old**

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

HPOT: The secretory epithelium appeared smaller than the controls, with small epithelial cells and no secretary material in the lumen.

**45 Day Old**

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

HPOT: The epithelium was well developed with prominent hypertrophied cells and the lumen was narrower than the controls. 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.

HPOT: The epithelium was well developed and highly convoluted, lined by hypertrophied cells. The lumen was full of secretory material.

**90 Day Old**

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

HPOT: The secretory epithelium was well developed, convoluted and lined by hypertrophied cells. The lumen was filled with secretory material.

## PLATE VI

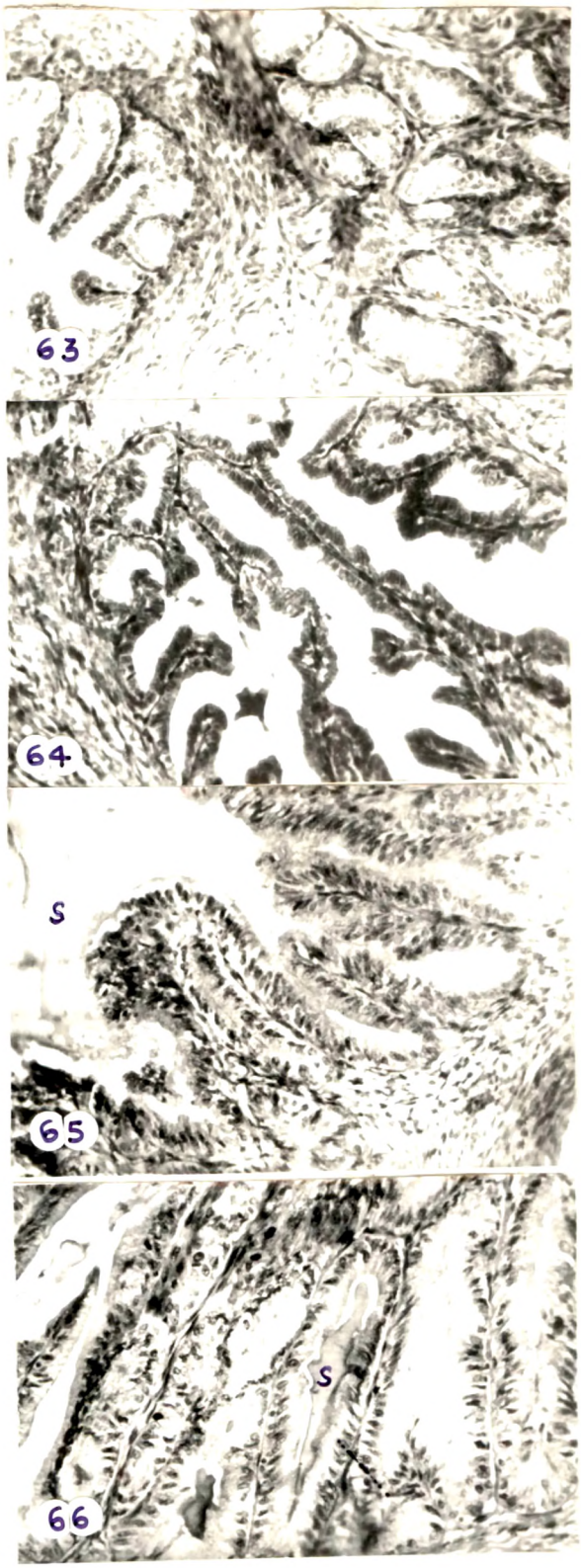
Figures 63 to 66- Photomicrographs of sections of seminal vesicles of 35, 45, 60, and 90 day old control rats (200 x).

Figure 63: Section of seminal vesicle of 35 day old rat showing small, less convoluted secretory epithelium.

Figure 64: Section of seminal vesicle of 45 day old rat showing better developed convoluted secretory epithelium.

Figure 65: Section of seminal vesicle of 60 day old rat showing well developed and convoluted secretory epithelium lined by columnar epithelial cells. Note the presence of secretory material (S) in the lumen.

Figure 66: Section of seminal vesicle of 90 day old rat showing very well developed highly convoluted secretory epithelium lined by tall columnar cells. Note the narrow lumen with secretory material (S).


63
64
S
65
S
66

## PLATE VII

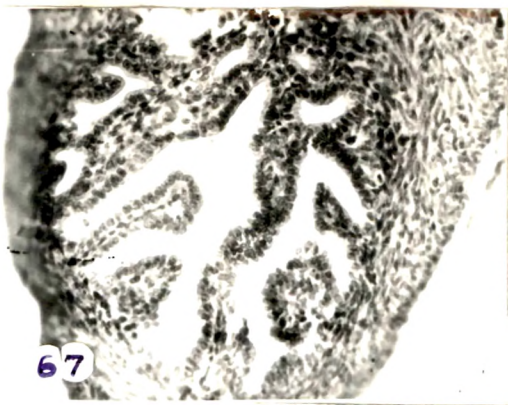
Figures 67 to 71- Photomicrographs of sections of seminal vesicle of 35, 45, 60, and 90 day old HPOT rats (200 x).

Figures 67 and 68: Sections of seminal vesicle of 35 day old rat showing less developed secretory epithelium with small epithelial cells.

Figure 69: Section of seminal vesicle of 45 day old rat showing better developed secretory epithelium with prominent hypertrophied cells with narrow lumen and secretory material (S) in it.

Figure 70: Section of seminal vesicle of 60 day old rat showing better developed and convoluted secretory epithelium lined by hypertrophied cells with secretory material (S) in the lumen.

Figure 71: Section of seminal vesicle of 90 day old rat showing prominent, convoluted secretory epithelium lined by hypertrophied cells.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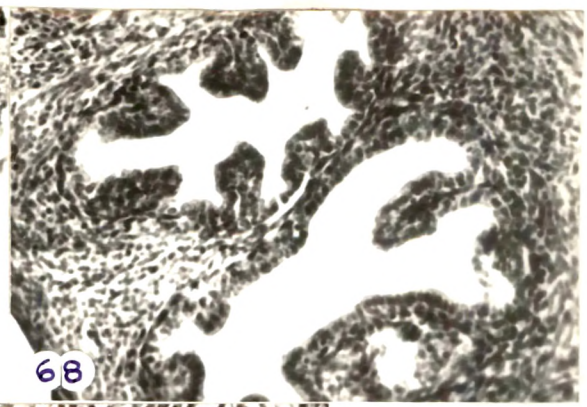
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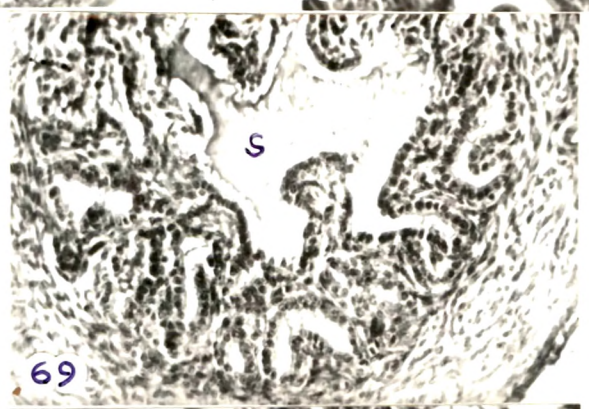
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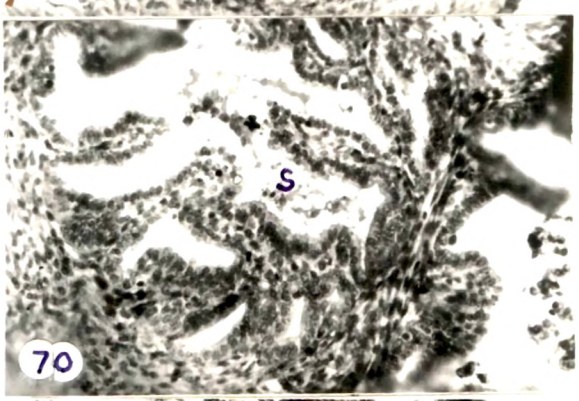
69


S
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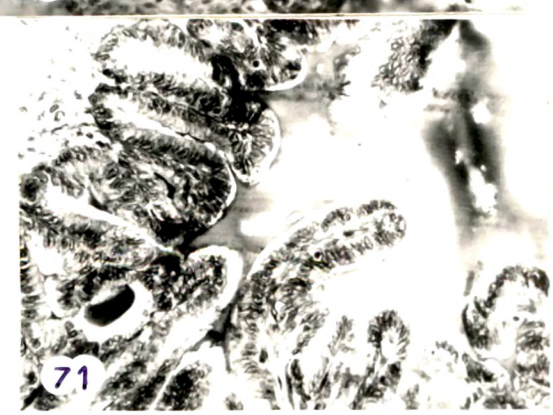

71

STRUCTURE OF PROSTATE (Plates VIII-X)

**35 Day Old**

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

HPOT: The acini were round and were lined by low cuboidal to large cuboidal epithelium.

**45 Day Old**

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

HPOT: The acini were well developed and lined by hypertrophied, cuboidal to columnar 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.

HPOT: The acini appeared more or less similar to that seen at 45 days but with increased size.

**90 Day Old**

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

HPOT: The acini were large and prominent and lined by cuboidal to columnar epithelium. The lumen was full of secretory material.

STRUCTURE OF THYROID (Plates XI & XII)

**35 Day Old**

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

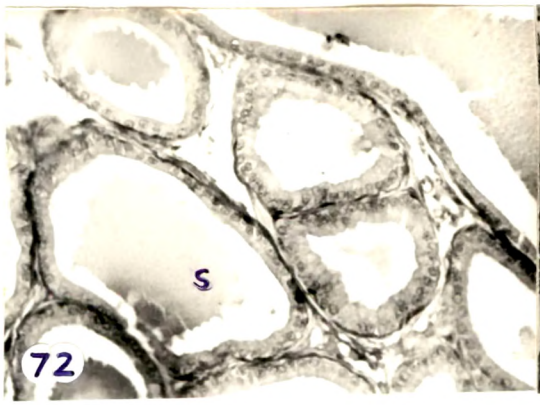
## PLATE VIII

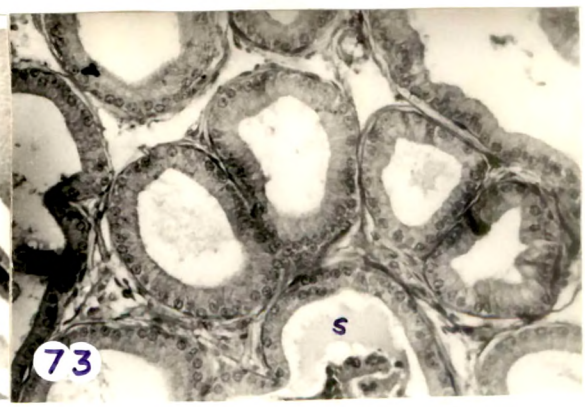
Figures 72-76: Photomicrographs of sections of prostate of 35,45 and 90 day old control rats (200.x).

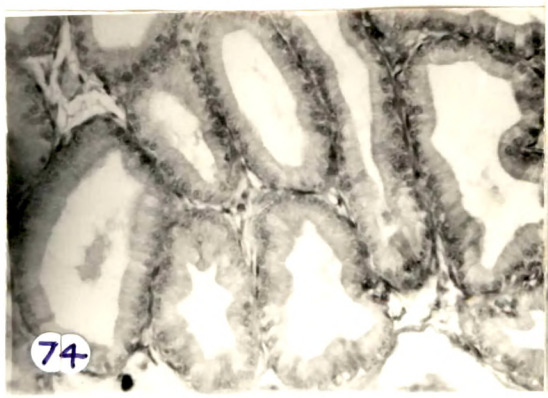
Figures 72 and 73: Sections of prostate of 35 day old rat showing less convoluted prostatic acini lined by cuboidal to columnar epithelial cells. Secretory material (S) is present in the lumen.

Figures 74and75: Sections of prostate of 45 day old rat showing well developed convoluted acini lined by tall columnar epithelium.

Figure 76: Section of prostate of 60 day old rat showing well formed acini lined by tall columnar cells and presence of amorphous secretory material (S).


S
72


S
73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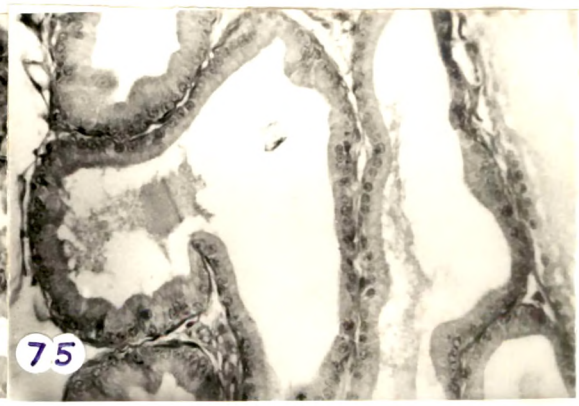
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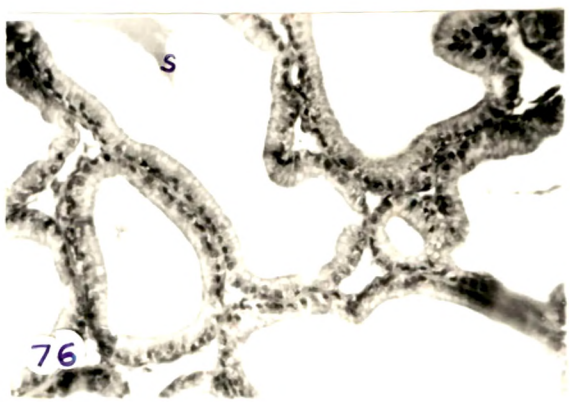
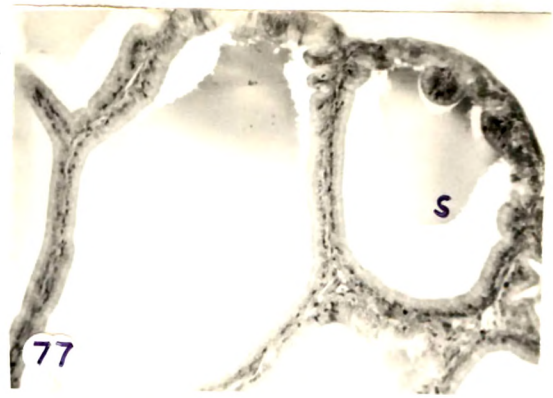

S
76

## PLATE IX

Figures 77-79: Photomicrographs of sections of prostate of 90 day old rats showing large prominent acini lined by tall columnar epithelial cells and secretory material (S) in the lumen (Fig. 77 and 78-100 x and Fig. 79- 200 x).


S
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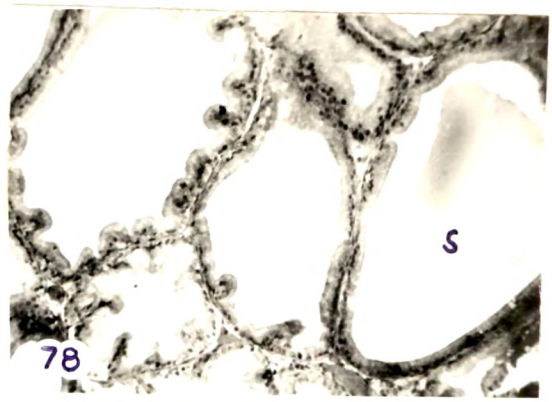
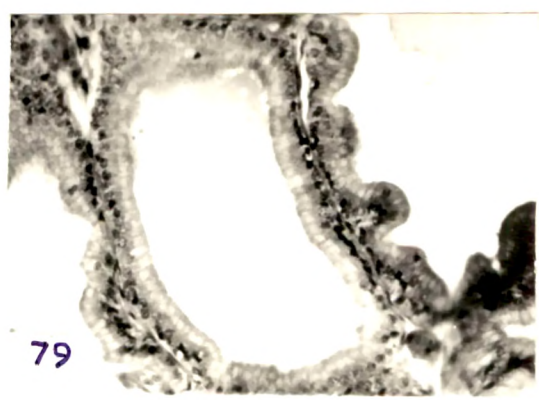

S
78


79

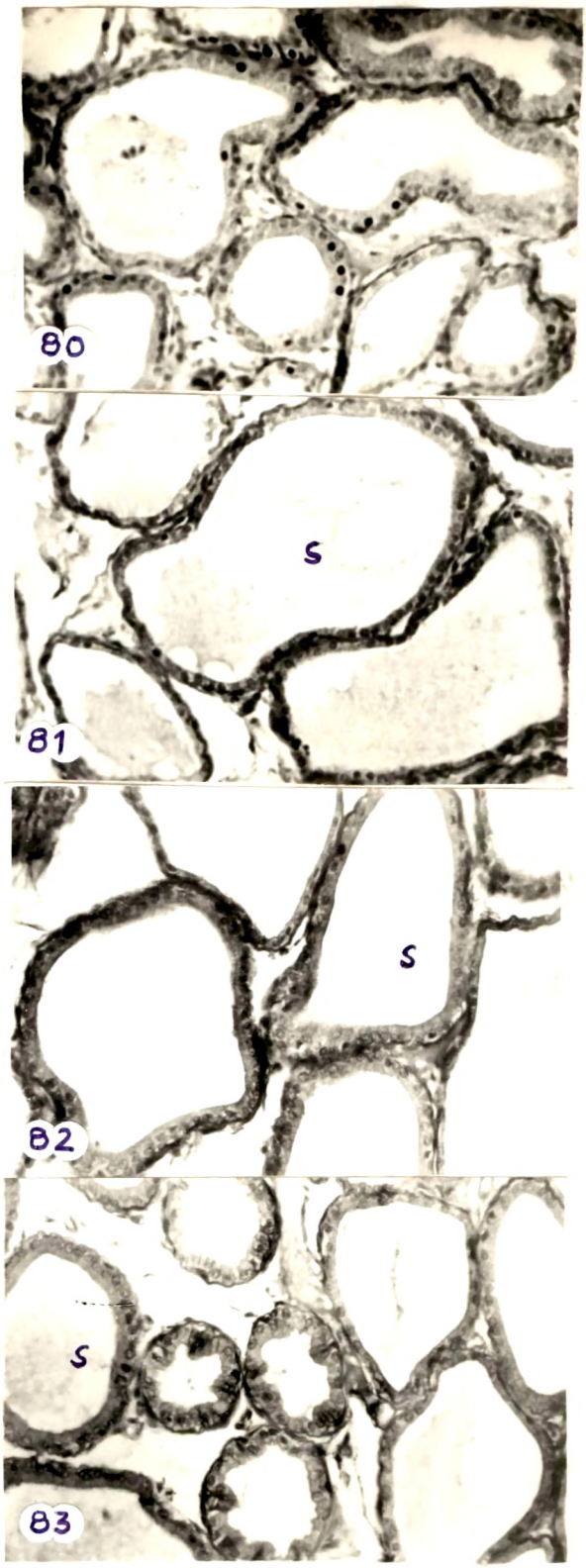
## PLATE X

Figures 80-83: Photomicrographs of sections of prostate of 35,45 and 90 day old HPOT rats (200.x).

Figures 80: Section of prostate of 35 day old rat showing round acini lined by low and large cuboidal epithelium.

Figure 81: Section of prostate of 45 day old rat showing well developed acini with secretory material (S)

Figures 82 and 83: Sections of prostate of 90 day old rat showing large acini lined by tall columnar to cuboidal epithelial cells and presence of secretory material (S) in the lumen


80
S
81
S
82
S
83

## PLATE XI

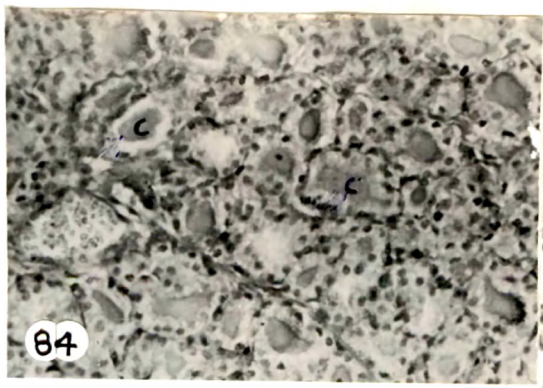
Figures 84-89: Photomicrographs of sections of thyroid gland of 35,45,60 and 90 day old control rats (200 x).

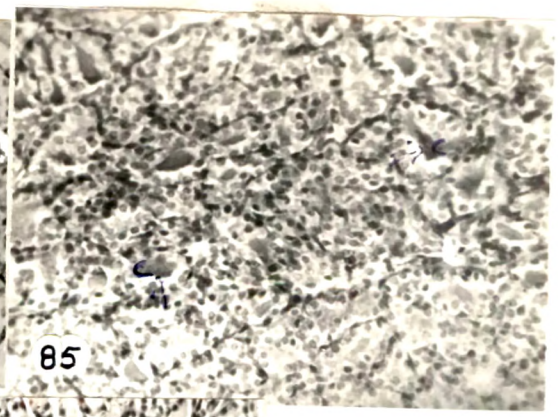
Figures 84 and 85: Sections of thyroid of 35 day old rat showing active follicles lined by large cuboidal cells with narrow lumen and little colloid (C) content

Figure 86: Section of thyroid of 45 day old rat showing less active follicles filled with colloid (C)

Figure 87: Section of thyroid of 60 day old rat showing follicles lined by hypertrophied epithelium and reduced colloid (C) content

Figures 88 and 89: Sections of thyroid of 90 day old rat showing normal follicles lined by cuboidal epithelium and filled with colloid (C)


C
C
84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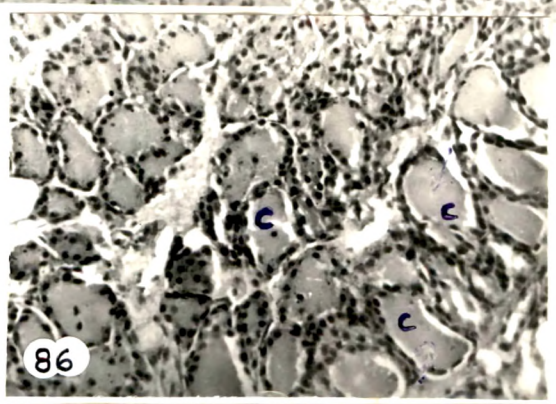
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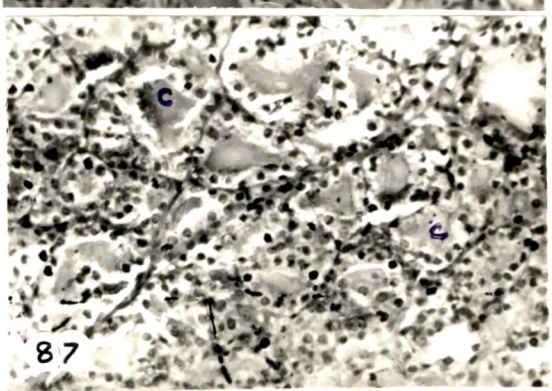
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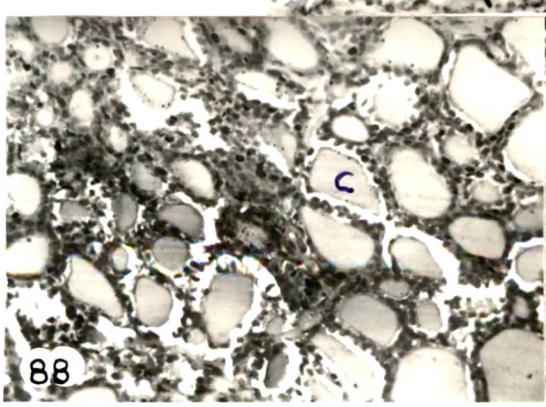
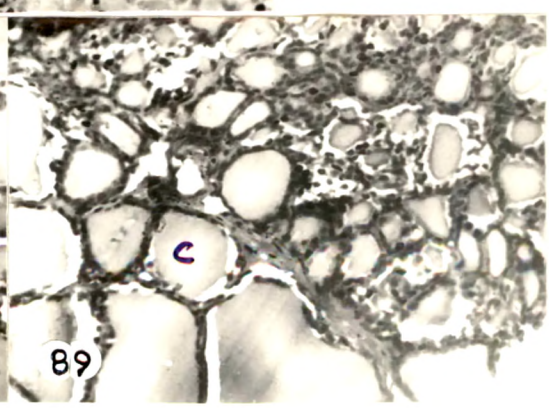

C
88


C
89

## PLATE XII

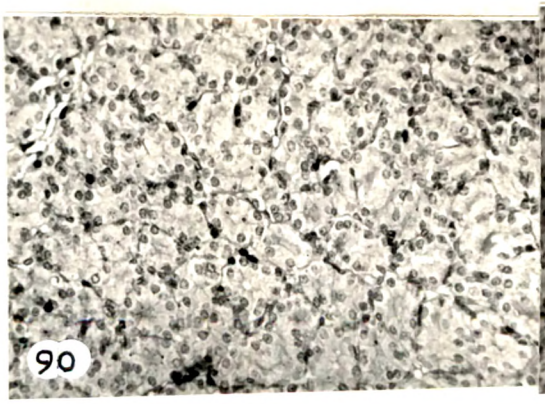
Figures 90-93: Photomicrographs of sections of thyroid of 35, 45, 60 and 90 day old HPOT rats (200 x).

Figure 90: Section of thyroid of 35 day old rat showing follicles lined by greatly hypertrophied cells with almost no lumen

Figure 91: Section of thyroid of 45 day old rat showing follicles lined by moderately hypertrophied and vacuolated cells. Narrow lumen visible with meagre colloid (C) content

Figure 92: Section of thyroid of 60 day old rat showing follicles lined by hypertrophied epithelium and narrow lumen with less colloid (C) content, but more than at 45 days

Figure 93: Section of thyroid of 90 day old rat showing follicles lined by cuboidal epithelium with prominent lumen and moderate colloid (C) content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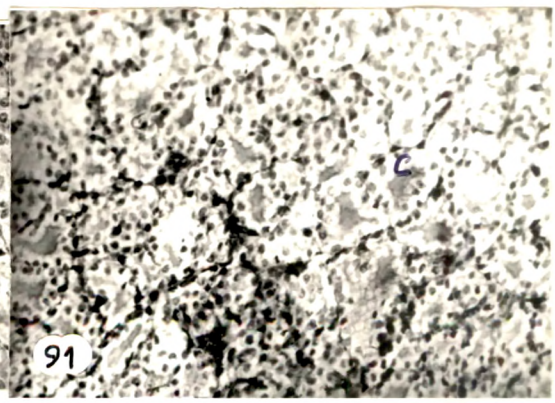
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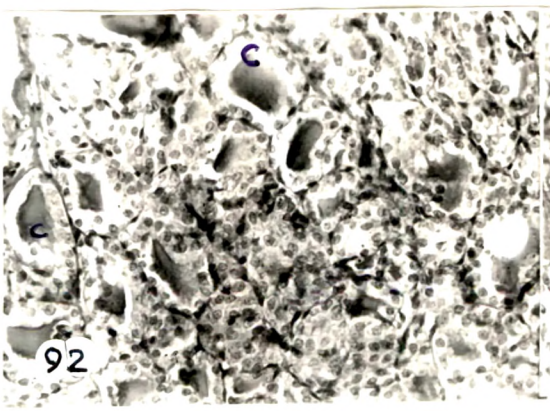
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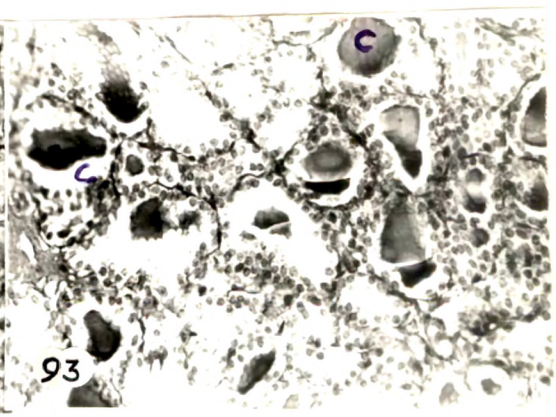
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HPOT: The follicular epithelium was greatly hypertrophied with the result the lumen was almost obliterated and there was no colloid.

**45 Day Old**

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

HPOT: The follicular epithelial cells were hypertrophied and vacuolated with narrow lumen and little colloid.

**60 Day Old**

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

HPOT: The follicular epithelium was hypertrophied and the follicles had narrow lumen and little colloid content.

**90 Day Old**

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

HPOT: The follicles were lined by cuboidal epithelium. Most of them showed moderate colloid content though, a few were empty.

**III. HISTOCHEMICAL OBSERVATIONS** (Plates XIII-XVII)

**35 Day Old**

3 ß-HSDH: In the testis of control animals, the enzyme activity was clearly discernible in the Leydig cells and, there was a weak localization in the tubules. The localization in the Leydig cells was discernible with DHEA as the substrate but not with pregnenolone (P). In the HPOT animals, the enzyme activity in the Leydig cells with DHEA as substrate was reduced and no activity was seen with P as substrate. However, the enzyme activity was more evident in the tubules with P.

17 ß-HSDH: The enzyme activity was appreciable in the tubules while no activity was visible in the Leydig cells. The HPOT animals also showed a similar pattern of enzyme activity.

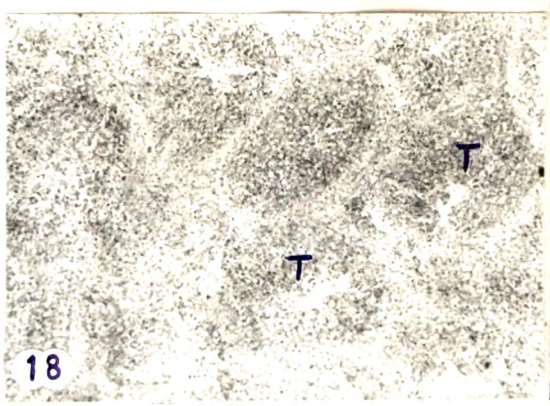
## PLATE XIII

Figures 18 to 23- Photomicrographs of sections of testis of 35, 45, and 60 days old control rats showing histochemical localisation of 3$\alpha$ HSDH and 17ß HSDH (65 x).

Figures 18 and 21: Sections showing week localisation of 3$\alpha$ HSDH in the tubules (T) (Fig. 18) and a slightly stronger localisation of 17ß HSDH also in the tubules (T) (Fig. 21) of 35 day old rat.

Figures 19 and 22: Sections of 45 day old rat showing mild but discernible activity of 3$\alpha$ HSDH in both the tubules (T) and interstitium (I) (Fig. 19) and strong localisation in the tubules (T) and week activity in the interstitium (I) of 17ß HSDH (Fig. 22).

Figures 20 and 23: Sections of 60 day old rat testis showing very strong localisation of 3$\alpha$ HSDH in the tubules (T) (Fig. 20) and localisation of 17ß HSDH in both the tubules (T) and the interstitium (I).



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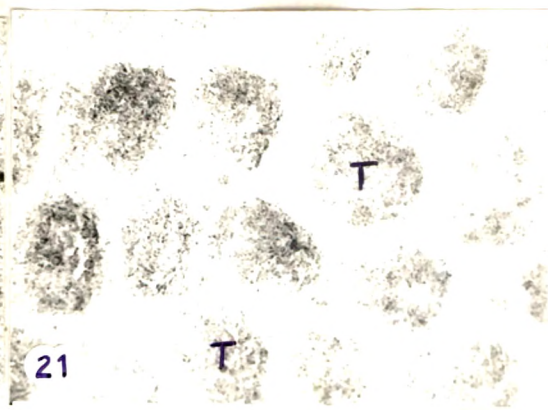

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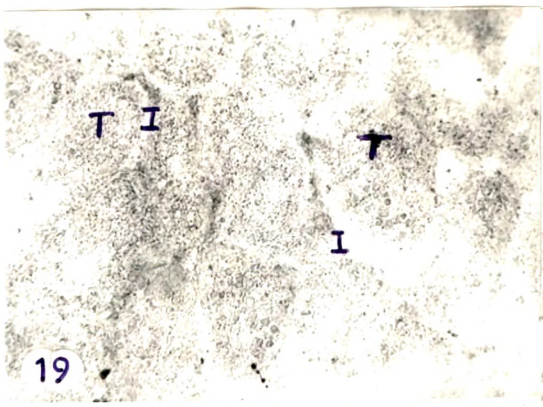

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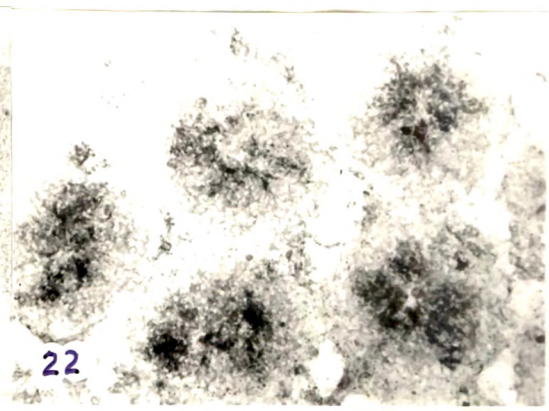
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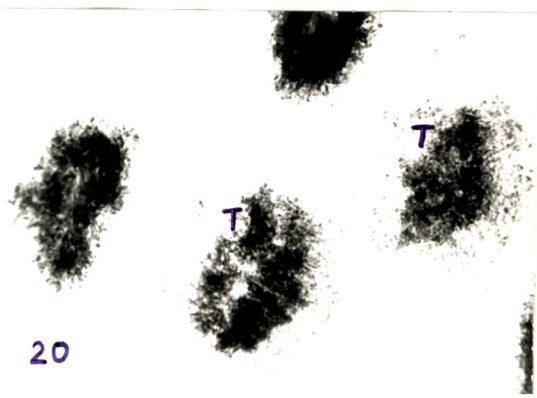

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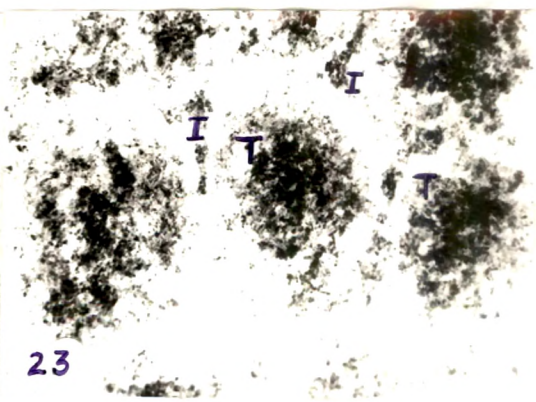

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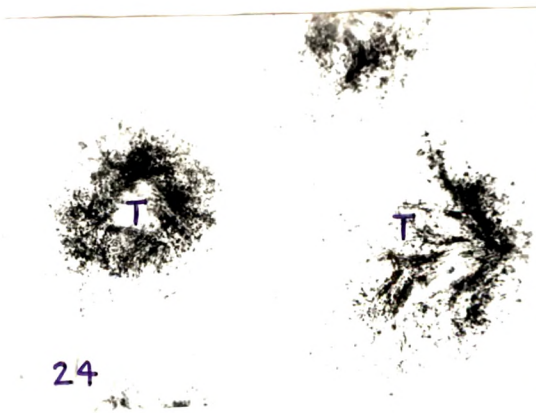
## PLATE XIV

Figures 24 to 27- Photomicrographs of sections of testis of 90 day old control rats depicting 3$\alpha$ HSDH and 17ß HSDH activities (65 x).

Figures 24 and 25: Sections showing the reduced 3$\alpha$ HSDH activity in the tubules (T) compared to 60 days and the more intense localisation in the central part of the tubules (T) containing the advanced stages of germ cells.

Figures 26 and 27: Sections showing localisation of 17ß HSDH. Note the discernible enzyme activity in the interstitium (I) and the strong localisation in the central part of the tubules (T) containing advance stages of germ cells.

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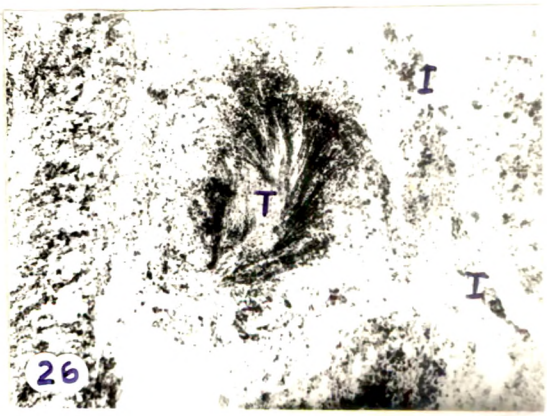


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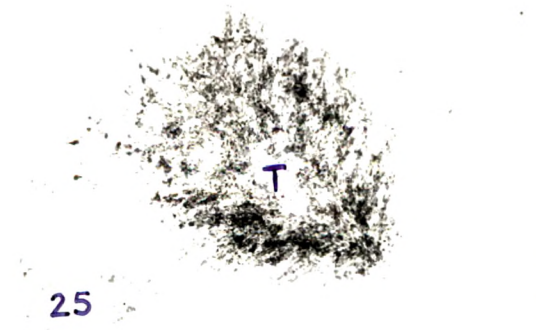


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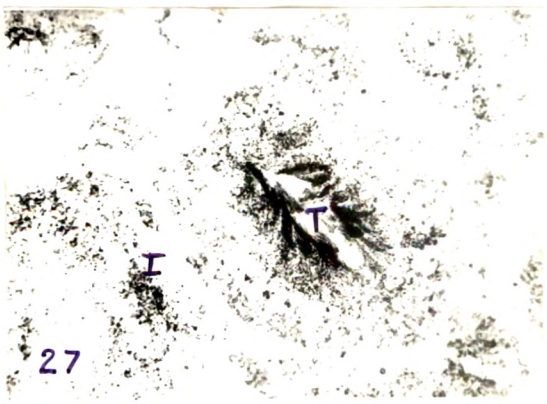


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## PLATE XV

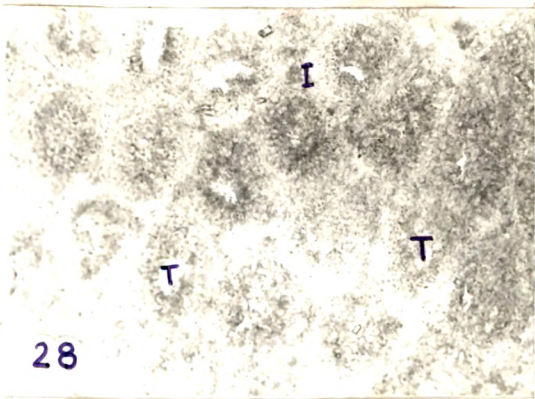
Figures 28 to 35- Photomicrographs of sections of testis of 35, 45, 60 and 90 day old HPOT rats demonstrating the localisation of 3$\alpha$ and 17ß HSDH (65 x)

Figures 28 and 32: Sections of 35 day old testis showing reduced 3$\alpha$ HSDH activity in the tubules (T) compared to the controls and mild activity in the interstitium (I) (Fig. 28) and noticeable 17ß HSDH localisation in the tubules (T) (Fig. 32).

Figures 29 and 33: Sections of 45 day old testis showing stronger 3$\alpha$ HSDH localisation in the tubules (T) with no activity in the interstitium (Fig. 29) and increased 17ß HSDH activity in the tubules (T) compared to the controls at 35 day (Fig. 33).

Figures 30 and 34: Sections of 60 day old testis showing weak 3$\alpha$ HSDH activity in the tubules (T) (Fig. 30) and noticeable 17ß HSDH activity in the tubules (T) and weak activity in the interstitium (I) (Fig.34).

Figures 31 and 35: Sections of 90 day old testis showing mild activity of 3$\alpha$ HSDH in the peripheral parts of the tubules (T) and the interstitium (I) (Fig. 31) and intense 17ß HSDH localisation in the central part of the tubules (T) containing advanced stages of germ cells (Fig. 35).

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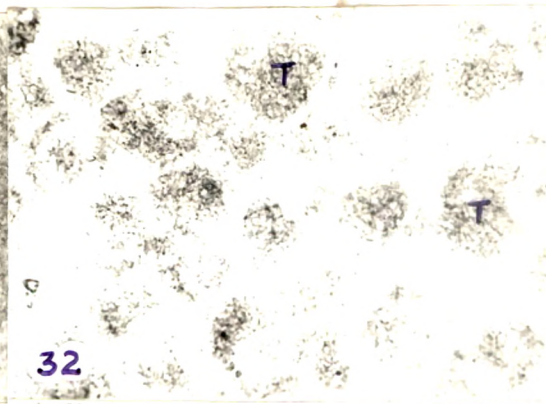


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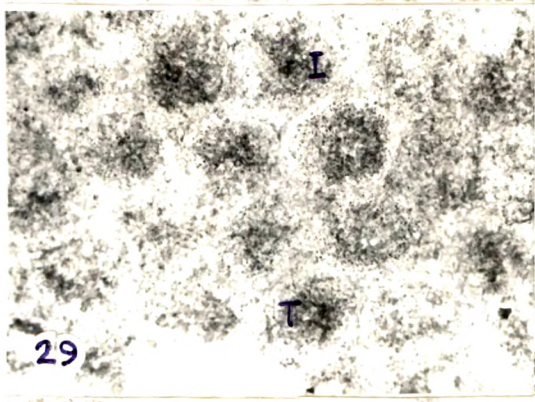


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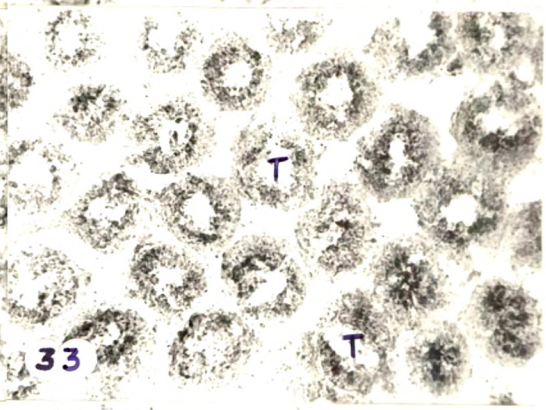


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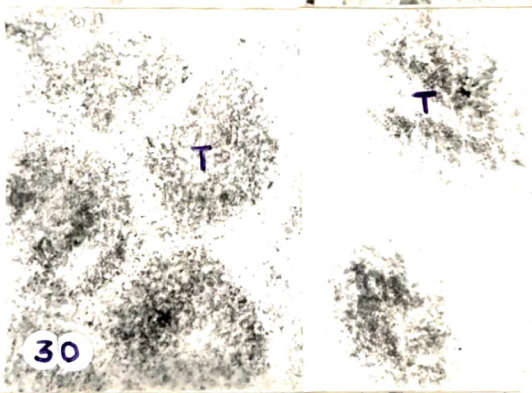


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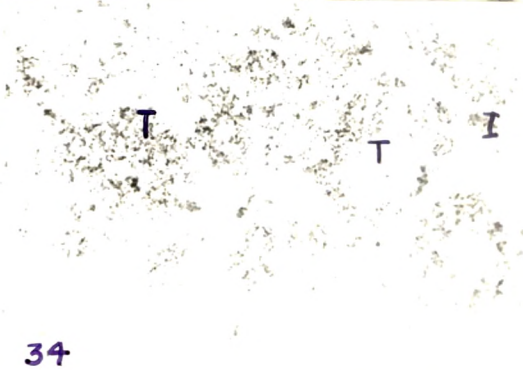


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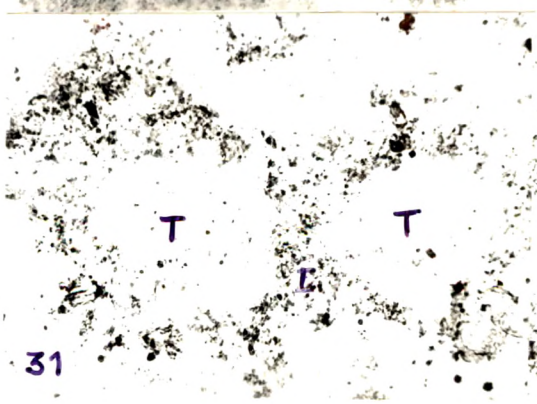


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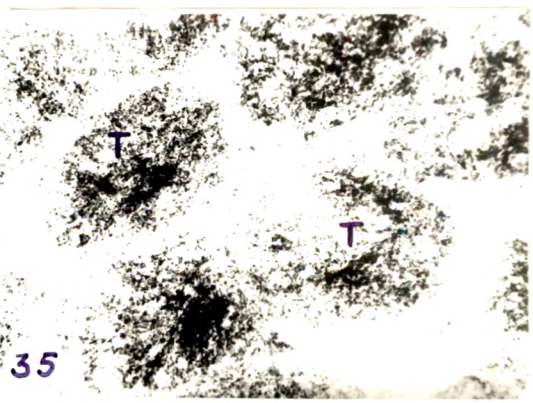


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## PLATE XVI

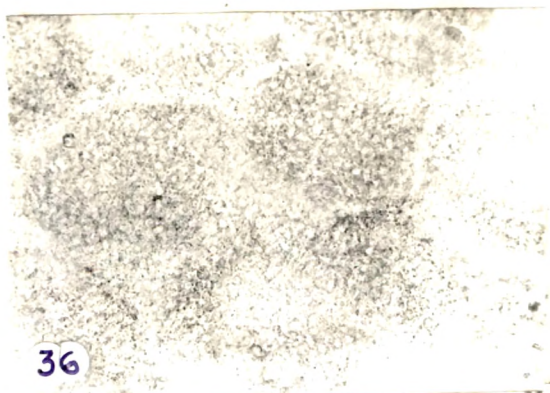
Figures 36 to 43- Photomicrographs of sections of testis of 35, 45, 60 and 90 day old control rat showing 3ß HSDH localisation with pregnenolone (P) and dehydro-epiandrosterone (DHEA) as the substrates (65 x).

Figures 36 and 40: Sections of testis of 35 day old rat showing discernible 3ß HSDH localisation in the interstitial (I) cells with DHEA as the substrate (Fig. 40)

Figures 37 and 41: Sections of testis of 45 day old rat showing reduced but noticeable 3ß HSDH localisation in the interstitium (I) with DHEA as the substrate (Fig. 41).

Figures 38 and 42: Sections of testis of 60 day old rat showing increased 3ß HSDH localisation in the tubules (T), more prominent with DHEA as the substrate (Fig. 42). Note the intense localisation of the enzyme in the central part of the tubules (T) containing advanced stages of germ cells and more uniform distribution in tubules (T) containing earlier stages.

Figures 39 and 43: Sections of testis of 90 day old rat showing reduced but noticeable enzyme activity in the tubules (T) and in the interstitium (I).The enzyme activity is noticeable more in those tubules (T) containing advanced stages of germ cells (fig. 39, P as the substrate and 43, DHEA as the substrate).



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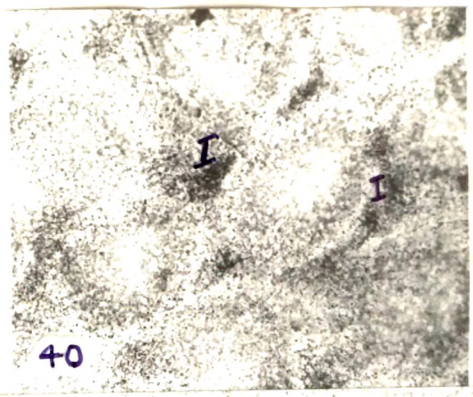


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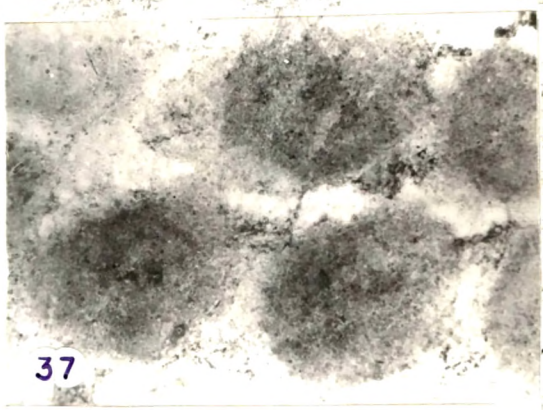

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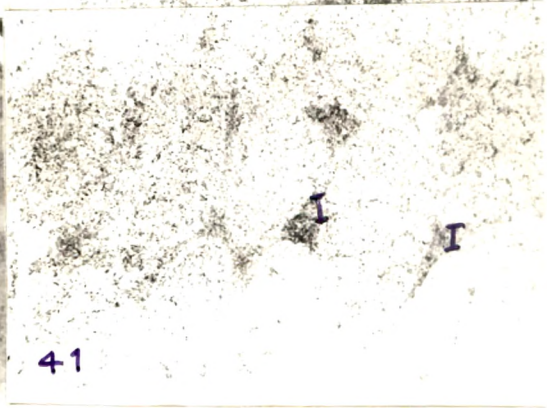


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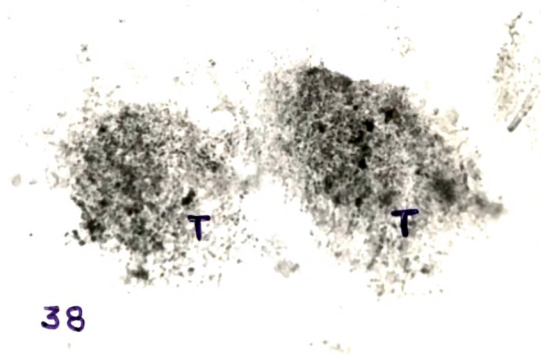


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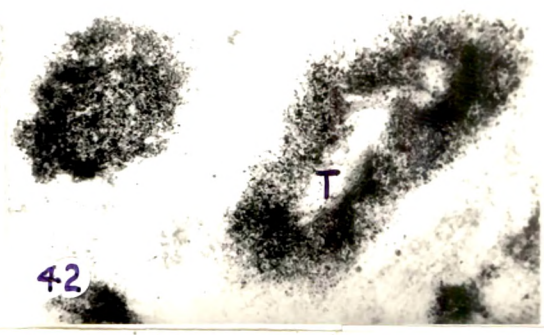


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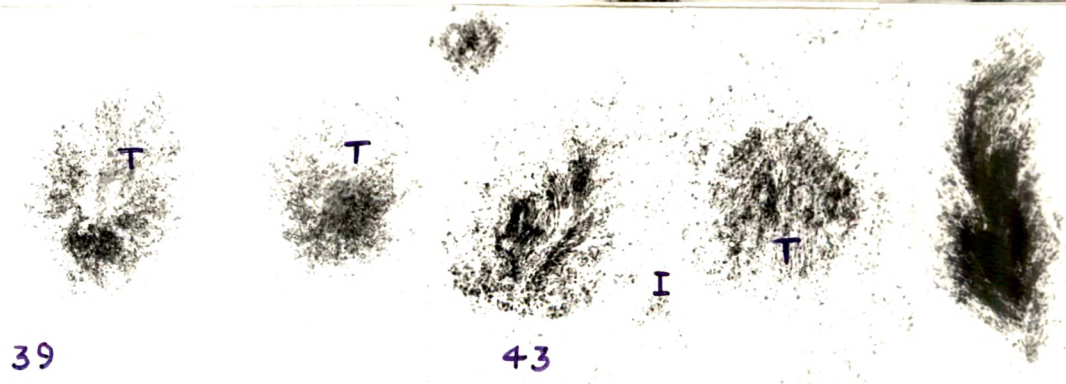


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## PLATE XVII

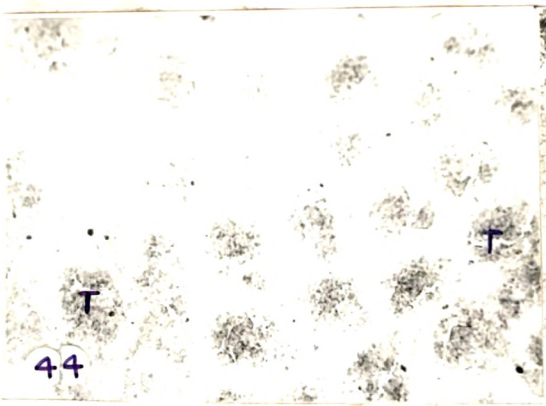
Figures 44 to 51- Photomicrographs of sections of testis of 35, 45, 60, and 90 day old HPOT rat showing 3ß HSDH activity with P and DHEA as substrates (65 x).

Figures 44 and 48: Sections of testis of 35 day old rat showing reduced 3ß HSDH localisation in the interstitium (I) with DHEA as the substrate compared to controls (Fig. 48) and noticeable enzyme activity in the tubules (T) with P as the substrate (Fig. 44).

Figures 45 and 49: Sections of testis of 45 day old rat showing mild activity in the interstitium (I) with DHEA as the substrate (Fig. 49) and positive enzyme activity in the tubules (T) with P as the substrate (Fig. 45).

Figures 46 and 50: Sections of testis of 60 day old rat showing strong localisation of the enzyme in the interstitium (I) with P and DHEA as the substrates and weak but noticeable response in the tubules (T).

Figures 47 and 51: Sections of testis of 90 day old rat showing mild localisation in the interstitium (I) with both the substrates and a more prominent peritubular (T) localisation with P as the substrate (Fig. 47).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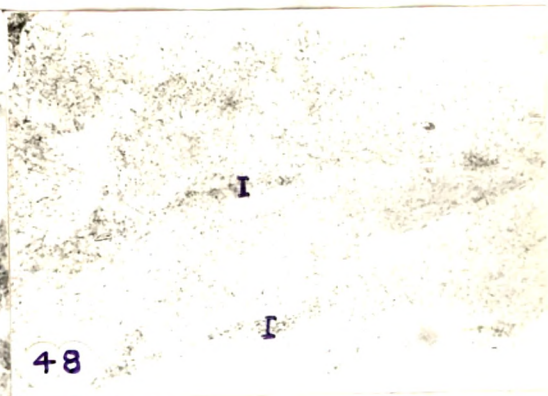
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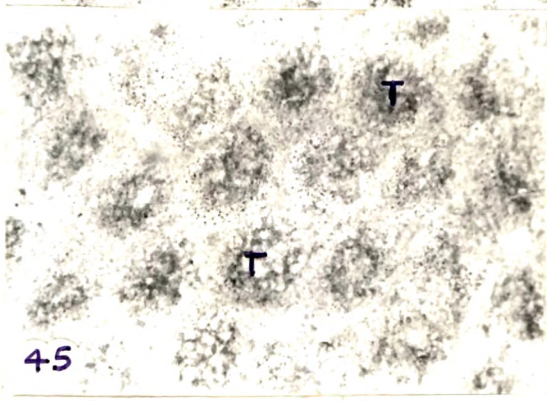
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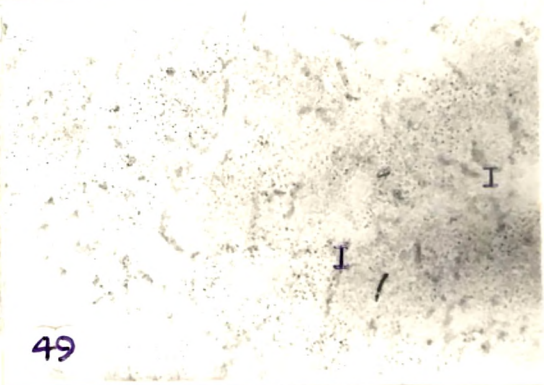
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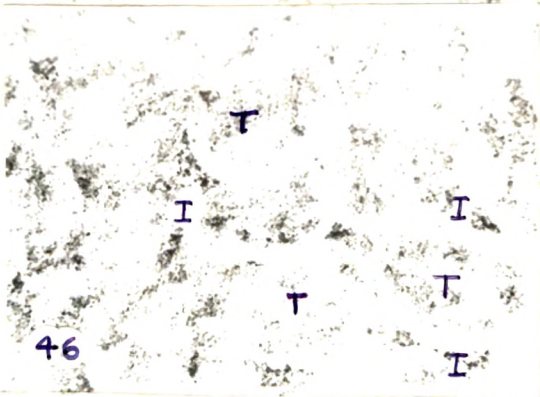
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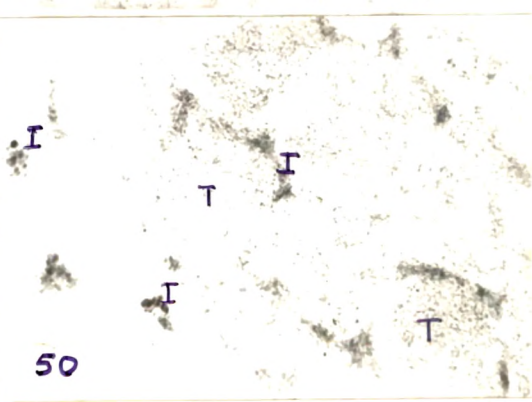
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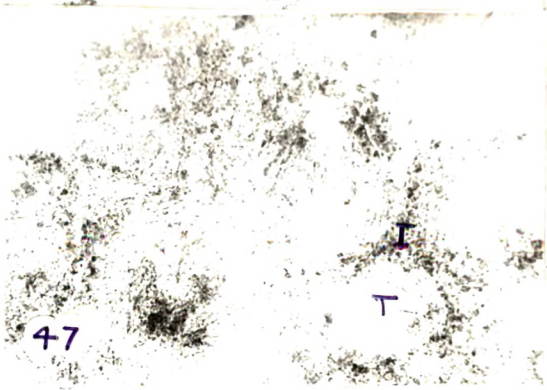
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3 $\alpha$-HSDH: The enzyme activity was weakly localized in the tubules but not in the Leydig cells of control rats. The HPOT animals also showed a similar but slightly reduced enzyme localization in the tubules while, the Leydig cells appeared to be relatively enzyme positive.

**45 Day Old**

3 ß-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. The tubules were enzyme positive with P as the substrate while, enzyme response with DHEA was very weak in HPOT animals.

17 ß-HSDH: In the control animals, the Leydig cells were weakly enzyme active while the tubules showed significant activity. Though the enzyme activity was localized uniformly within the tubules containing early stages of germ cells, the enzyme activity was localized more in the luminal part in tubules containing advanced stages of spermatogenesis. In the HPOT animals, the enzyme activity was intense in the tubules while, it was weak in the interstitium. The enzyme activity appeared to be increased as compared to the 35 days.

3 $\alpha$-HSDH: The enzyme activity was mild though discernible in tubules as well as in the interstitium of the control animals. The enzyme activity was strongly localized in the tubules while there was no noticeable activity in the interstitium in the HPOT animals.

**60 Day Old**

3 ß-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 HPOT rats, the interstitium showed strong

localization and the tubules weak response with DHEA as substrate. However, with P as substrate, the tubules and the Leydig cells were more enzyme responsive.

17 ß-HSDH: 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 tubules of HPOT animals showed strong enzyme activity while the Leydig cells showed mild activity. Compared to 45 days, the enzyme activity was lesser.

3 *α*-HSDH: In the control, the enzyme activity was very strongly localized in the tubules, almost as intense as 3ß-HSDH activity. In HPOT animals the tubules showed weak but noticeable enzyme activity and was lesser than that at 45 days and it was more like the 45 day controls.

**90 Day Old**

3 ß-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 localized 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 HPOT rats, the enzyme activity was reduced as compared with 60 days and, the activity was peritubular with P as the substrate and, mild to moderate activity with both DHEA and P as substrates in the interstitium.

17 ß-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 HPOT rats, the distribution of the enzyme activity was very much like in the controls, however, the enzyme activity was less intense and appeared more like the 60 day controls.

3 *α*-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 the HPOT animals,

there was no enzyme activity in the tubules except a mild response in the peritubular part. Leydig cells were enzyme positive.

**IV. SERUM HORMONE PROFILE** (Table 1.6; Fig. 12 a, b & c)

**$T_3$ and $T_4$**

Both $T_3$ and $T_4$ increased continuously in control rats from 35 days to reach peak levels at 60 days and then decreased by 90 days to the 35 day level. The HPOT animals had significantly low $T_3$ and $T_4$ levels at 35 days and their levels increased gradually to reach the characteristic adult levels between 60 and 90 days.

**Testosterone**

The serum T level increased in the control rats to peak level at 45 days. At 60 days the T level was reduced but then again increased to a higher level by 90 days, slightly less than the 45 day level. The HPOT animals showed persistently low levels of T till 60 days, though increasing very slowly, to attain near adult levels by 90 days. In fact, a significant increase was noticed between 60 and 90 days.

## DISCUSSION

Over the years, a mediatory influence of thyroid hormones on reproductive functions in mammals, including man, has been realised. Hypothyroidism in man has been reported to affect male reproductive functions in general though, variable effects have been manifested, depending on the age of onset of HPOT as well as etiology (Jannini *et al.*, 1995). Studies involving experimental induction of HPOT in male rats had revealed differential effects. Vilchez-Martinez (1973), observed no effect on the weight, structure and functions of testis and accessory glands in adult rats rendered hypothyroidic, though some decrease in pituitary-gonadotropin contents were noted. Other workers showed that though fertility is not effected, the reproductive system organ weights were affected by adult HPOT (Jones *et al.*, 1946; Karkun *et al.*, 1967; Baksi, 1973; Bruni *et al.*, 1975; Kalland *et al.*, 1978). Even differential effects on serum levels of

Table 1.6 Chronological alterations in Serum Triiodothyronine, Thyroxine and Testosterone levels in intact and hypothyroid (HPOT) 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 |
| HPOT | 1.53 ± $0.02^{d}$ | 1.84 ± $0.03^{d}$ | 1.81 ± $0.03^{c}$ | 2.50 ± $0.04^{ns}$ | 27.75 ± $1.88^{d}$ | 43.10 ± $2.48^{d}$ | 54.00 ± $2.95^{d}$ | 62.00 ± $3.14^{c}$ | 0.41 ± $0.19^{ns}$ | 0.46 ± $0.11^{d}$ | 0.58 ± $0.17^{ns}$ | 0.90 ± $0.20^{b}$ |

@ Values expressed as Mean ± SD of five experiments

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

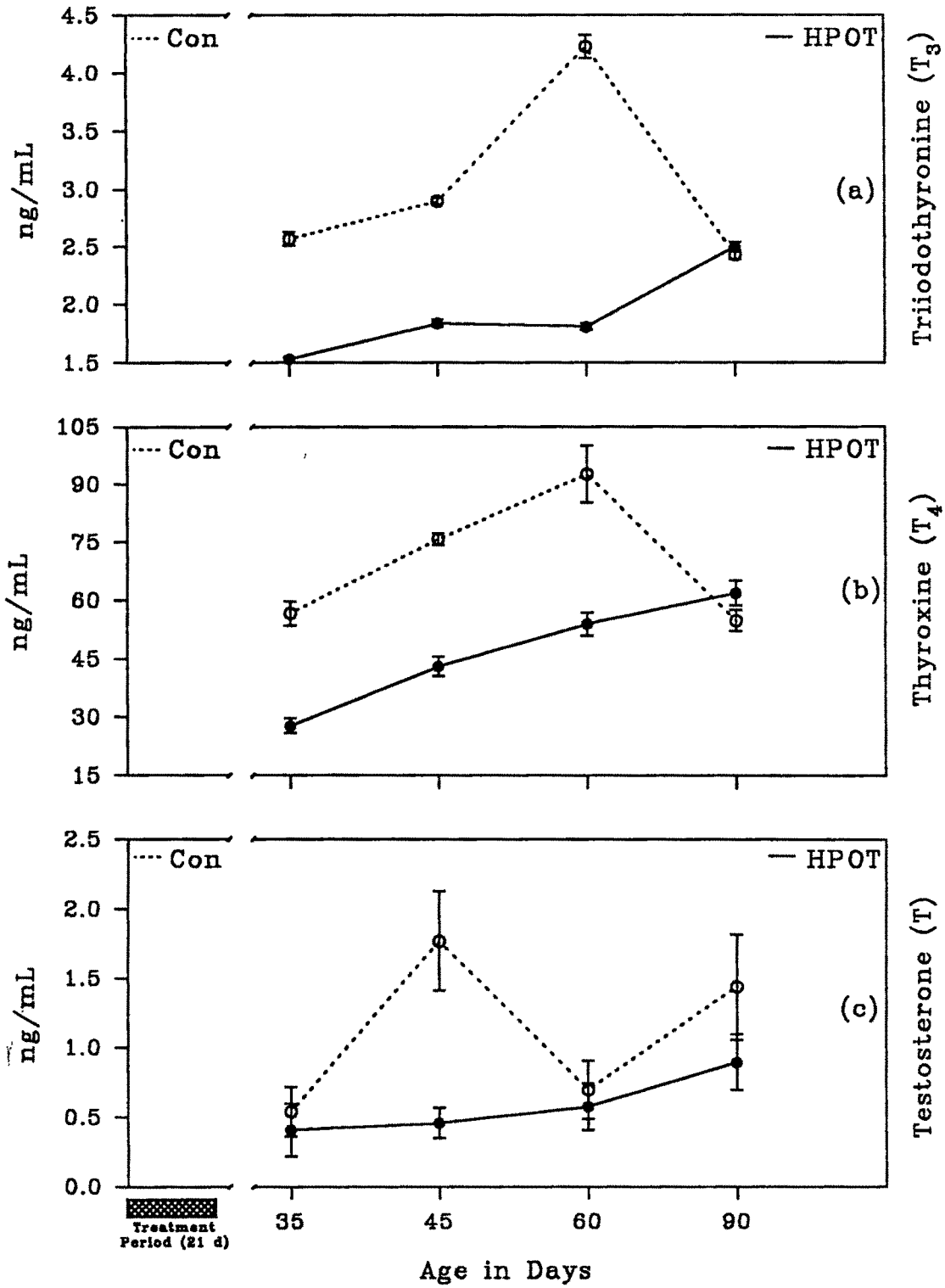


Fig. 12 (a, b & c) Chronological alterations in serum $T_3$, $T_4$ and T levels in intact and hypothyroid (HPOT) rats

gonadotropins and testosterone (T) were also reported. Whereas Baksi (1973) reported lower serum LH but no difference in FSH and Bruni *et al* .(1975), showed lowered serum levels of FSH, LH and T, Kalland *et al* .(1978), obtained no difference in the basal serum levels of any of the three hormones. In a later study conducted on 40 day old male Wistar rats showed that, HPOT significantly impaired body growth, reduced total testicular weight and induced atrophy of sex accessory glands (Valle *et al.*, 1985). They also showed decreased serum levels of FSH, PRL and T and reduced responsiveness of Leydig cells to LH and also lower intra-testicular T levels. Though these changes were reversed by $T_3$ administration and, normalised the serum hormone levels, the body and sex accessory gland weights were not fully corrected. The above work showed that the reproductive system of immature rat is more sensitive to thyroid dysfunction than that of the adult animals.

Recent works on transient HPOT induced in the neonatal period (upto weanling) have observed paradoxical hypertrophy and plasticity of the testis in the post-weanling recovery period, with a nearly doubled testicular size in the adult stage (Cooke and Meisami, 1991; Meisami *et al.*, 1992). The increased adult testis size attained due to neonatal hypothyroidism has also been shown to result in increased sperm production (Cooke *et al.*, 1991; Cooke *et al.*, 1993), which has generated optimism that this could be a model of choice for increasing the reproductive potential in economically productive animals.

Paradoxically, similar experimental protocol employed in the present study has failed to reproduce the above results. In the present study, the neonatal rats subjected to transient HPOT during the preweanling period (to be referred to as HPOT henceforth) showed a constant significantly reduced testis weight between 35 and 60 days. The difference in weight compared to the controls remained between 80 to 90%. This is very evident as the growth rate was marginal between 35 and 45 days and nil between 45 and 60 days in the HPOT rats while, the controls showed a continuous increase in growth rate from 35 day onwards with a peak rate

being attained between 45 and 60 days. The present study shows a close parallelism between body weight and testis weight changes. The peak increment in body weight as well as testis weight in the control animals occurred between 45 and 60 days. Both these parameters showed peak compensatory increment between 60 and 90 days in the case of HPOT rats. In striking contrast to the observations of Cooke and Meisami (1991) and Meisami *et al* .(1992) of the HPOT rats lagging behind the controls in body weight at the end of 180 and 210 days respectively, the body weight of HPOT rats in the present investigation equalled that of the controls by 90 days itself. Though the body weight difference was totally nullified by 90 days, the testis weight still showed a deficit of 30%. This is despite the fact that the testes weight showed a peak growth rate between 60 and 90 days amounting to a gain in weight of 1083%. Previous workers had obtained a peak compensatory growth in HPOT rats between 55 to 75 days during which the testes weight overtook that of the control by 70 days itself. In neither of the earlier works (Cooke and Meisami, 1991; Meisami *et al.*, 1992) did further increase in testicular weight between 75 and 180 or 210 days exceed 40%. Considering this, it is very clear that further growth of testes in our experimental animals after 90 days can at best equal the controls as, the deficit in testes weight at 90 days was still 30%. Though the serum $T_4$ and $T_3$ levels appear to be slightly higher in our control rats, the levels of the hormones in the HPOT rats during the recovery period were very much comparable to those reported by the previous workers and attained control levels by 60 days in both cases. Besides, the histological observations of thyroid gland also confirmed the induction of hypothyroidism. Our unpublished observations of testes weight at 120 days as well as the present histological observations bear testimony to this.

The histological observations revealed that the testis of HPOT rats had smaller tubules and impaired spermatogenesis. Whereas spermatogenesis was fully established in the testis of control animals between 45 and 60 days, marked by the appearance of spermatids by 45 days and sperms by 60 days, the same was delayed in HPOT rats and, a quantitatively and quantitatively poor spermatogenic process occurred only by 90 days. In keeping with this, the

maximal increase in tubular diameter also occurred at these periods in control and HPOT animals. Germ cell degeneration was very much prominent in HPOT animals at 35 days and persisted till as late as 60 days. Some degree of recovery denoted by arrest of degeneration of germ cells could be seen only by 45 days. The serum thyroid hormone profile reveals that the period of germ cell degeneration corresponds to HPOT state with sub-normal serum $T_4$ and $T_3$ levels. A possible explanation for the observed germ cell degeneration in the HPOT animals could be the enhanced formation of estradiol in the Sertoli cells as demonstrated by Panno *et al.* (1994). At 60 days, the testis of HPOT animals depicted differential effects with some tubules still showing degenerating germ cells and some tubules showing recovery and establishment of spermatogenesis. Though spermatogenesis was established by 90 days, there were quantitatively lesser number of germ cells and, the sperm density appeared to be less compared to the controls. These histological observations are corroborated by the low serum T level and the pattern of histochemical localization of 3ß and 17ß-HSDH in the testis of HPOT rats in relation to the controls. The lower circulating titre of T till 60 days is understandable in the light of histochemically weak 3ß and 17ß-HSDH in the interstitium, though 3ß-HSDH activity became noticeable by 60 days. In contrast, the activity of these enzymes was well expressed in the interstitium of the controls animals by day 45. As evidenced, steroidogenic activity in the interstitium was clearly established in HPOT rats only by 90 days. The reasons for the reduced enzyme activity and low T levels could be the reduced serum gonadotropin levels (Kirby *et al.*, 1992; Van Haaster *et al.*, 1992; Simorangkir *et al.*, 1995) as well as the reduced sensitivity of the Leydig cells. The latter aspect finds justification from a recent report demonstrating reduced levels of LH and FSH and diminished activities of 3ß- and 17ß-HSDH in the Leydig cells of rats rendered hypothyroidic in the prepubertal period (Antony *et al.*, 1995). These workers further demonstrated reduced sensitivity of the Leydig cells to LH under *in vitro* conditions and concluded that prepubertal hypothyroidism suppresses both basal and LH stimulated Leydig cell activity. A very interesting feature that emerges from the histochemical observations is the intra-

tubular androgenesis which could be seen even at 35 days when it was incomplete in the interstitium. Though this was evident in both control and HPOT animals, there was a striking difference in the pattern. Whereas, the control testis showed distinct compartmentalisation with 3ß-HSDH activity in the interstitium and 17ß in the tubules, the testis of HPOT animals showed prominent localization of both the enzymes in the tubules. Though both the control and HPOT animals tended to show an active $\Delta^5$ pathway, there was co-operative interaction between the two components in the controls, with the androstenedione (A) required for the conversion to T in the tubules being provided by the Leydig cells. But in the HPOT animals, this appeared to be essentially an intra-tubular phenomenon. By 45 days, the control animals also depicted a full activity of intra-tubular steroidogenesis but, compared to the HPOT animals, the preferred pathway appeared to be the $\Delta^4$. By 60 days, steroidogenesis by both the pathways was fully established in the tubules and the Leydig cells of the control animals. In the HPOT animals, increased T production by the $\Delta^5$ pathway appeared operative in the tubules and the steroidogenic potential of the Leydig cells became apparent by the representation of 3ß-HSDH activity. A major difference evident is that, while the Leydig cells of control animals depicted steroidogenic potential by both the pathways, the Leydig cells of HPOT animals seemed to depict a preferential $\Delta^5$ pathway till 60 days. Localization of both the enzymes in the tubules, apparently in the Sertoli cells, denote intra-tubular steroidogenic potential, however insignificant it might be in the context of the overall T production by the testis. Apparently, intratubular steroidogenesis and steroid metabolism is of some significance and as such this was demonstrated even previously (Mehan *et al.*, 1989). This aspect, though less reported in the literature, has found validity from past studies (Dufau *et al.*, 1971). Some conclusions that could be drawn from the present observations are:

- In the control animals, Sertoli cell generation of T seen at 35 days can synergize with FSH and thyroid hormone in inducing Sertoli cell differentiation, and regulate spermatogenesis. This is supported by the presence of androgen receptors in Sertoli cells

(Bremner *et al.*, 1994) and the up-regulation of androgen receptors by $T_3$ in peripubertal rats (Panno *et al.*, 1996). Since hypothyroidism up-regulates estrogen receptors and down-regulates androgen receptors (Panno *et al.*, 1996), the Sertoli cell generated testosterone in the hypothyroid rats at 35 days could result in higher pool of free testosterone which could be detrimental for germ cell survival as seen presently.

- With the full establishment of spermatogenic functions, the disappearance of 3ß- and 17ß-HSDH activities from all tubules except for those populated by spermatids and spermatozoa in both control and HPOT animals, suggest an important role for Sertoli cell generated T in the final stages of spermatogenesis. Validity for this is provided by the reported importance of T in regulating spermatogenesis and more specifically in maintaining VII-VIII and IX-XIV stages of spermatids (see, Kerr *et al.*, 1992). Further, T is also implicated in maintaining the physical adhesion between the Sertoli cells and spermatids by controlling Sertoli cell ectoplasmic specialisations, and formation of adhesion molecules in spermatocytes and spermatids. This is clearly illustrated by the observed rampant degeneration of spermatocytes and spermatids in the lumen of the tubules, consequent to T withdrawal or absence (see, McLachlan *et al.*, 1996).

- Neonatal hypothyroidism seems to channelize testicular steroidogenesis solely through the $\Delta^5$ pathway with the total exclusion of the $\Delta^4$ pathway, unlike in the controls where, both the pathways become operative. Possible role of thyroid hormone in regulating the expression of $\Delta^4$ specific isozymic form of 3ß-HSDH in the neonatal period could be hypothesized and needs further evaluation.

The morphometric and histological alterations in the accessory sex glands reflect to a certain extend the changes seen with reference to the gonadal functions. The weights of epididymis, seminal vesicle and ventral prostate remained at an average 76 to 85% less than the controls between 35 and 60 days of age. In the control rats, all the three accessory organs

showed maximal growth increment between 45 and 60 days. However, in the HPOT rats, all the three organs showed hardly any growth till 60 days but between 60 and 90 days there was significant recovery denoted by very high percentage of growth increase. Nevertheless, their weight were still only 65 to 70% of the control weights.

Histologically, the epididymal tubules, the seminal vesicle secretory epithelium as well as the prostatic acini showed prominent growth in HPOT rats only between 60 and 90 days. The same occurred in control animals between 45 and 60 days. The marginal increase in weight observed between 35 and 60 days in HPOT rats seems to be chiefly due to hypertrophy of the cells as evidenced by the histological profile. Considering the observed morphometric and histological differences between 35 to 60 days in the control and HPOT animals, it is presumable that the growth of these organs in the neonatal and prepubertal periods is dependent on an adequate level of thyroid hormones and PRL. As noted in the present study, as well as from the reports of other studies, both these hormones were suppressed in the HPOT animals during the neonatal and prepubertal periods (Valle *et al.*, 1985; Kirby *et al.*, 1992). In the pubertal and post-pubertal periods, the growth and maturation of these organs are apparently controlled by both PRL and T. Recent reports have highlighted the importance of PRL in the somatogenic growth of prostate and has also proved that, androgens alone cannot induce full growth and maturation of the prostate gland (Reiter *et al.*, 1995a, b). In fact, synergistic action of PRL and T in controlling growth and functions of the accessory glands and especially prostate have been reported (Kharroubi and Slaunwhite, 1984). Though the recovery growth spurt shown by the HPOT animals between 60 and 90 days is the consequence of normalized T and PRL levels, the clear deficit in weight shown by the HPOT animals suggest that probably a deficiency of thyroid hormone at a critical neonatal period may result in a sub-normal sensitivity of these organs to T and PRL. Some evidence for this is provided by the observations of Valle *et al.*(1985) of normalisation of PRL and T levels but not the weight of sex accessory glands in immature male HPOT rats supplemented with $T_3$. This has been considered probably due to the depressed

peripheral response to PRL at the receptor level caused due to hypothyroidism (Fagilia *et al.*, 1980., Padron *et al.*, 1981).

On the whole, the observations of the present work indicate, no doubt, a delayed recovery growth of testis and accessory glands in HPOT rats, but never surpassing the control weights as observed by previous workers (Cooke and Meisami, 1991; Meisami *et al.*, 1992). A careful consideration of these apparently anomalous observations does not seem to be contradictory as the strains of rats used by the other workers were the Long-Evans and the Sprague-Dawley while, the present one is Charles-foster strain. Obviously, there is an inherent genetically determined strain difference in terms of the impact and the degree of response of the male reproductive system to neonatal hypothyroidism. In this connection, there are reported differences in the time schedule of expression of androgen receptors in prostate (Shanuon and Cunha, 1983; Takeda *et al.*, 1985; Husmann *et al.*, 1991; Takeda and Chang, 1991; Prins and Birch, 1995). The discrepancies have been attributed to the different strains of rats used (Prins and Birch, 1995) and as such strain effects on steroid receptor ontogeny in the reproductive tract have been reported (Cunha *et al.*, 1991). Other studies on certain inbred strains of mice have also shown differential sensitivity of cultured Leydig cells with respect to the inhibitory effect of T on basal 3ß-HSDH activity which, is essentially mediated through androgen receptors (Saez, 1994).

Apart from the observed difference in the response of the testes and the accessory glands, certain other differences evident compared to other strains used are (1) smaller adult body weight, (2) normalization of the body weight in the hypothyroidic individual by 90 days, and (3) a conspicuous decrease in the body weight, and the weights of testes and accessory glands at 45 days.

Since, the hypertrophy of the testes in the other two strains has been attributed to a prolonged phase of Sertoli cell proliferation and the consequent increase in the total number of

Sertoli cells and germ cells in the testes, it does not seem to be happening in our strain and it would be worthwhile to evaluate these aspect in Charles-foster strain as well.