CHAPTER - VII

Evening melatonin nullifies the effects of neonatal hypocorticalism on adult germ cell number and degeneration in male rats.

Short-term influences of melatonin in immature rats have been evaluated by a few workers. Some of these works have shown diminished ovarian and uterine weights (Wurtman *et al.,* 1963; Motta *et al.,* 1967) and retarded testes and accessory sex organ development (Debeljuk, 1969; Kinson and Robinson, 1970; Kinson and Peat, 1971). A dose dependent inhibitory effect on sexual maturation due to exogenous melatonin administration in the afternoon at 12-14 days of age has also been reported (Lang *et al.,* 1983). In contrast, they reported no influence of melatonin during the prepubertal period between 5-20 days or even in the adult period between 70 to 90 days (Lang *et al.,* 1983). Melatonin excess during the period of 20-45 days of age resulted in a delay of sexual maturation of male rats, but with no later effects on sexual functions (Lang *et al.,* 1983). Previous study from this laboratory showed a time independent inhibitory influence of melatonin on body and testes weights in the neonatal period (Patel and Ramachandran, 1992). The above study has indicated a definite

influence of melatonin in neonatal period on body and organ growth as well as on the development of reproductive system and on metabolic functions. In continuation with the above study, long-term effects of melatonin administered in the neonatal period were evaluated in terms of body and testes weights, testis histoarchitecture and serum hormone profiles (Chapter 1). Apart from melatonin effect on body and testes growth rate as well as on the set points of various hormonal axes, increased germ cell number but coupled with paradoxical germ cell degeneration, were the observations made in the above study.

In another study, the effect of neonatal hypocorticalism was also evaluated as a corollary to observations made with reference to neonatal hypercorticalism (Chapter 6). This study revealed decreased germ cell number with greater degree of germ cell degeneration as against increased germ cell number with reduced apoptosis and degeneration in corticosterone treated rats (Chapters 2 & 3).

A further study on evening melatonin in conjunction with hypercorticalism showed potentiating effect of melatonin on germ cell degeneration (Chapters 4 & 5). Hence, it was decided to test the consequent effect of combined hypermelatonemia and hypocorticalism. To this end rat neonates treated with metyrapone and administered with melatonin in the evening, were used to assess the body and testes growth rates, hormonal

profiles and testis histoarchitecture & functions in the adult, as long-term effects.

MATERIALS AND METHODS:

Animal and Maintenance:

As in chapter one.

Preparation of Metyrapone:

As in chapter six.

Experimental Protocol:

The experimental set-up was divided into two major groups of study, both consisting of subgroups as mentioned below.

Group I (control) **(C)**:

Newborn rat pups maintained till 90 days served as controls. This consisted of 3 subgroups (as follows) of 30 animals each:

(i) Control rats **(N)**

(ii) Injected *i.p.* with vehicle (0.9% saline) in morning (0800 hrs) + evening (1600 hrs).

Group II (Metyrapone + Melatonin) **(MET+MT)**:

Zero day old rat neonates were injected with metyrapone in the morning (0800 hrs) in the following doses:

0.5 mg metyrapone/animal/day from day 0 to day 10 and

1.0 mg metyrapone/animal/day from day 11 to day 21 and melatonin in the evening at 1600 hrs (40μg melatonin/animal/day from day 0 to day 21 post partum)

Parameters and Methods of Evaluation:

As in chapter one.

Histology and Histometry:

As in chapter one.

Hormone Assays:

As in chapter one.

Statistical Analysis:

As in chapter one.

RESULTS:

Since no significant difference was observed between vehicle and non-vehicle controls, the data represented is of vehicle control **(C)** only.

Postnatal Growth:

The body and testes growth rate of MET+MT rats at 90 days was lower than the controls though the body weight was slightly higher at 45 and 60 days. The body growth rate shows continuous increment in control animals with peak growth rate at 45-90 days but in MET+MT animals, significant higher growth rate was expressed between 35-60 days. Similarly, the growth rate of testes showed significant increment in the experimentals between 35-45 days, at all other periods the growth rate in experimental animals was lesser than that of the controls (Table 2; Figures 3a & 3b). The relative weight of testes was significantly lower at all ages with maximal effect at 35 days (Table 1; Figure 2).

Histology and Histometry:

The sections of testis of MET+MT animals showed loosely packed germ cells with many degenerating and apoptotic cells at 35 days. By 45 days there was an increase in the number of germ cells with reduced number of degenerating and apoptotic cells. The lumen of tubules showed the presence of degenerating elongating spermatids, and premature release from germinal epithelium. At 60 and 90 days, the apoptotic or degenerating cells were greatly reduced and there was increased number of germ cells and spermatozoa in the tubules. The sperm density at 90 days appears to be comparable to controls. The Leydig cells appeared quite compact and

The data of:

- Body weight and Testes weight
- Relative weight
- Body growth rate and Testes growth rate
- Corticosterone level
- LH and T levels
- TSH level
- T_4 and T_3 levels

of treatments MT and MET has been recalled from chapters 1 and 6 respectively.

Table 1: Chronological alterations in body weight (g) and absolute (g) and relative weight (g/100 g) of testes in Control and Metyrapone + Melatonin treated rats

Treatment	Body Weight					Testes Weight					Relative Testes Weight				
	Age in days					Age in days					Age in days				
	15	35	45	60	90	15	35	45	60	90	15	35	45	60	90
C	34.75 ±1.942	82.0 ±2.804	122.5 ±3.677	197.33 ±6.396	360.9 ±6.280	0.215 ±0.019	0.86 ±0.050	1.346 ±0.058	2.603 ±0.085	3.586 ±0.104	0.67 ±0.034	1.056 ±0.060	1.108 ±0.062	1.399 ±0.074	0.986 ±0.05
MET + MT	29.26 ±2.64	86.0 ±3.89	154.7^{c} ±4.678	239.5^{c} ±6.894	344.0^{a} ±6.781	0.161 ±0.026	0.661^{a} ±0.096	1.601 ±0.146	2.518 ±0.078	3.402 ±0.063	0.57^{c} ±0.016	0.724^{c} ±0.024	1.377^{a} ±0.09	1.153 ±0.12	0.890 ±0.08
* C	37.33 ±1 99	85.573 ±1.902	117.16 ±2.307	193.66 ±5.493	322.4 4.078	0.218 ±0.017	0.803 ±0.061	1.28 ±0.09	2.28 ±0.08	3.067 ±0.102	0.755 ±0.002	0.944 ±0.025	1.09 ±0.040	1.178 ±0.073	0.933 ±0.01
MT	31.15 ±1.417	110.33 ±2.490	152.33 ±2.486	233.66 ±3.242	396.66 ±9.898	0.213 ±0.003	0.938 ±0.054	2.214 0.102	2.214 ±0.102	3.375 ±0.078	0.683 ±0.001	0.854 ±0.004	1.11 ±0.42	1.16 ±0.063	0.847 ±0.04
* C	34.75 ±1.942	82.0 ±2.804	122.5 ±3.677	197.33 ±6.396	360.9 ±6.280	0.215 ±0.019	0.86 ±0.050	1.346 ±0.058	2.603 ±0.085	3.586 ±0.104	0.67 ±0.034	1.056 ±0.060	1.108 ±0.062	1.399 ±0.074	0.986 ±0.05
MET	31 0 ±1.864	100.0^{a} ±6.164	175.0^{c} ±3.106	258.3^{c} ±6.540	354.16 ±5.665	0.179 ±0.013	0.86 ±0.034	1.913^{c} ±0.048	3.173^{c} ±0.052	3.480 ±0.052	0.58 ±0.014	0.86 ±0.024	1.091 ±0.056	1.228 ±0.068	0.982 ±0.04

C – Control, **MET + MT** - Metyrapone+ Evening Melatonin injection, **MET** – Metyrapone, **MT** - Evening Melatonin injection

Values expressed as Mean ± SEM of six animals. ^{a} $p < 0.05$, ^{b} $p < 0.005$, ^{c} $p < 0.0005$

* Values recalled from Chapters 1and 6

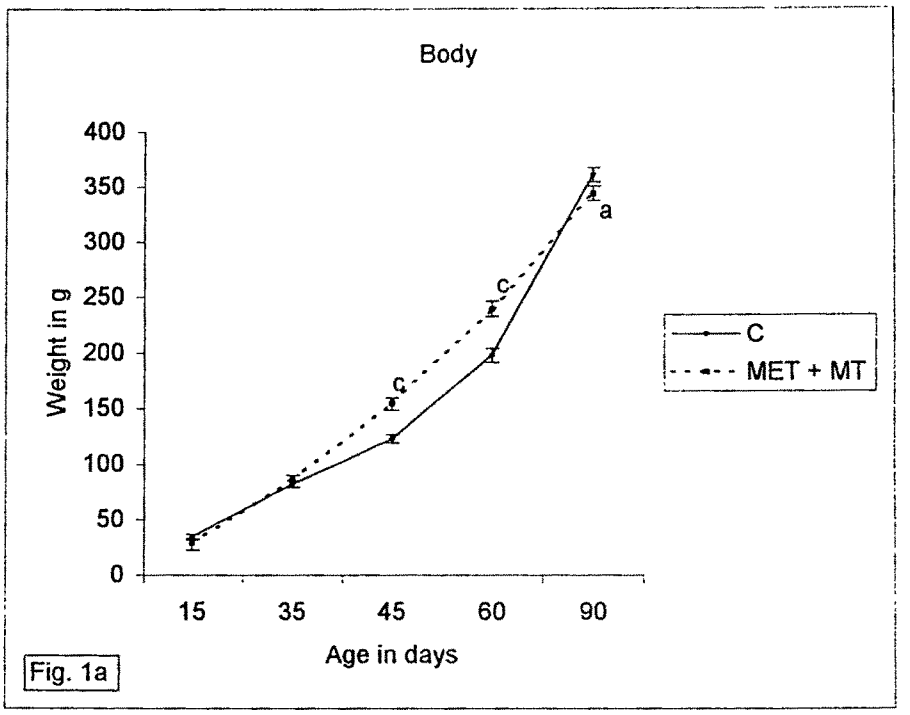


Fig. 1a

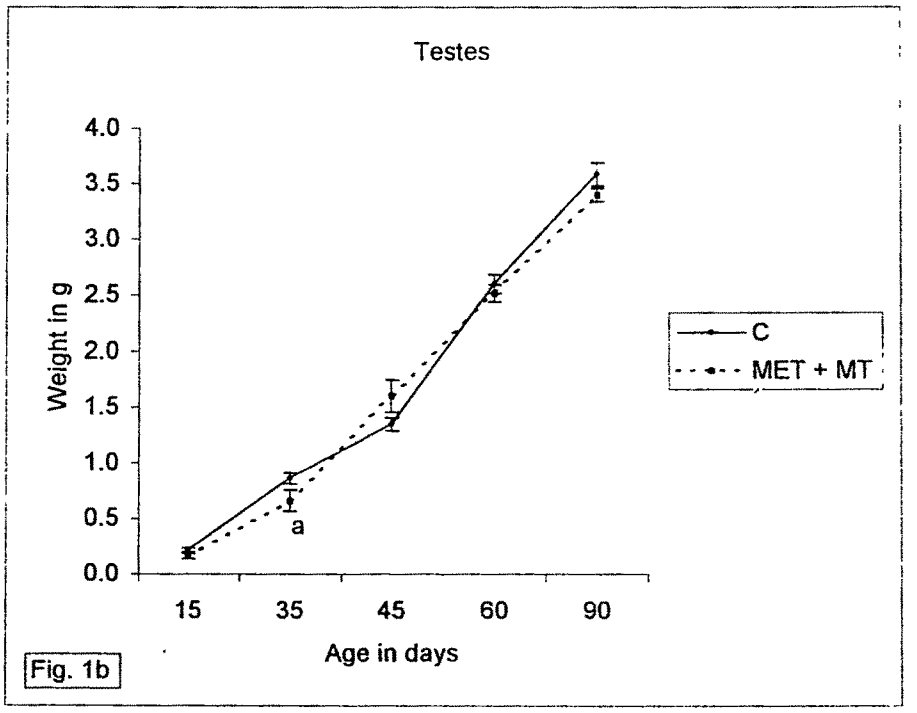
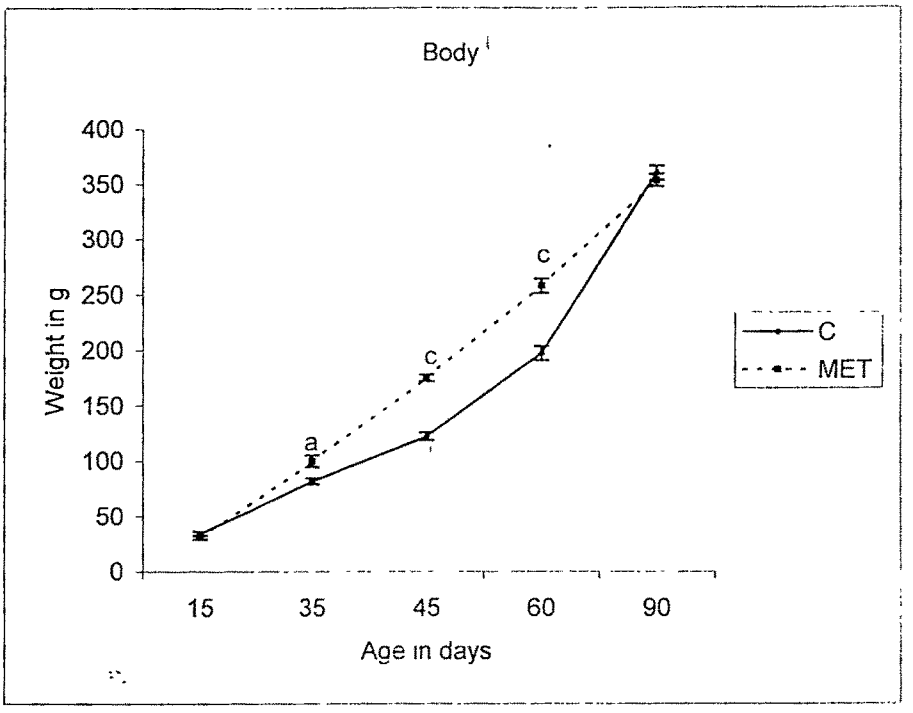
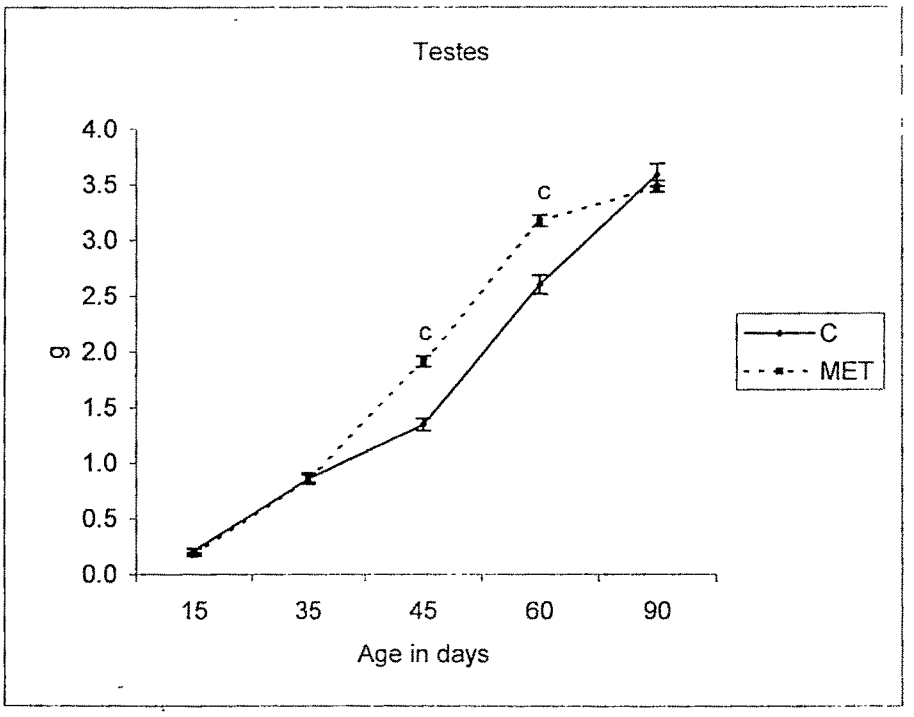


Fig. 1b

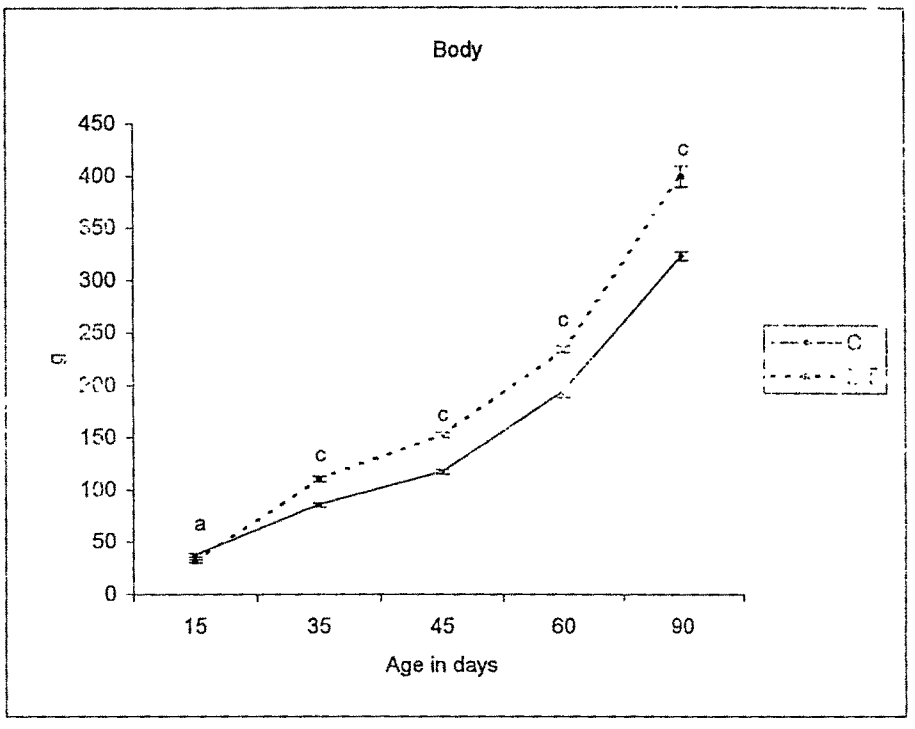
Fig. 1a and 1b: Chronological alterations in body weight (g) and absolute weight (g) of testes in Control and Metyrapone + Melatonin treated rats
C - Control, **MET+MT** - Metyrapone+ Melatonin treated rats
Values expressed as Mean ± SEM of six animals
a $p < 0.05$, b $p < 0.005$, c $p < 0\ 0005$

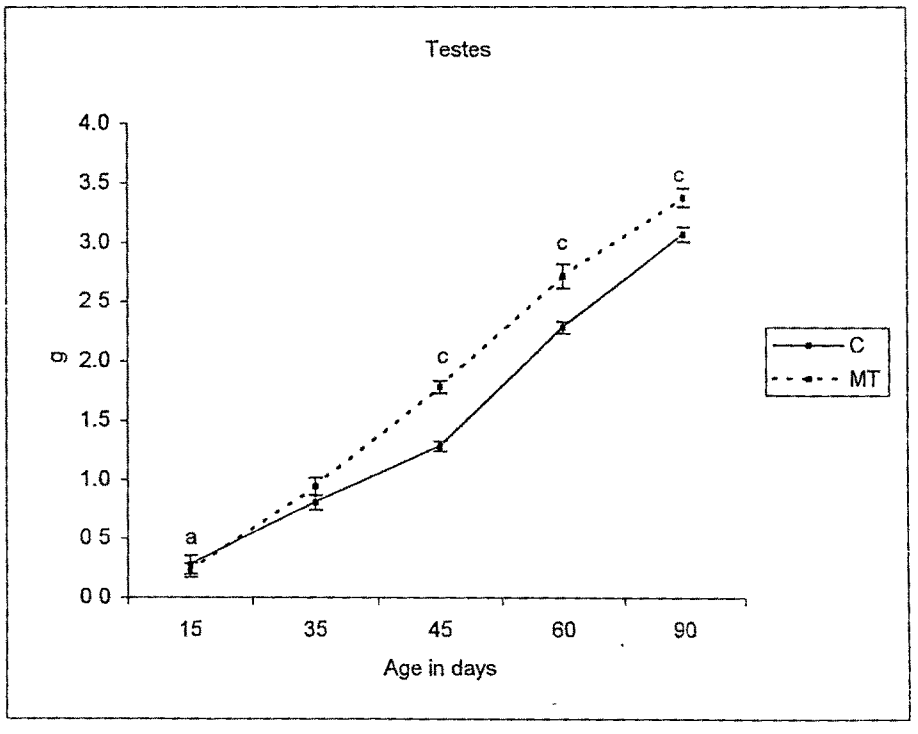




Chronological alterations in body weight (g) and absolute weight (g) of testes in Control and Metyrapone treated rats

C - Control, **MET** - Metyrapone, Values expressed as Mean ± SEM of six animals, [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$





Chronological alterations in body weight (g) and absolute weight (g) of testes in Control and Melatonin treated rats

C – Control, MT – Melatonin treated

Values expressed as Mean ± SEM of six animals [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$

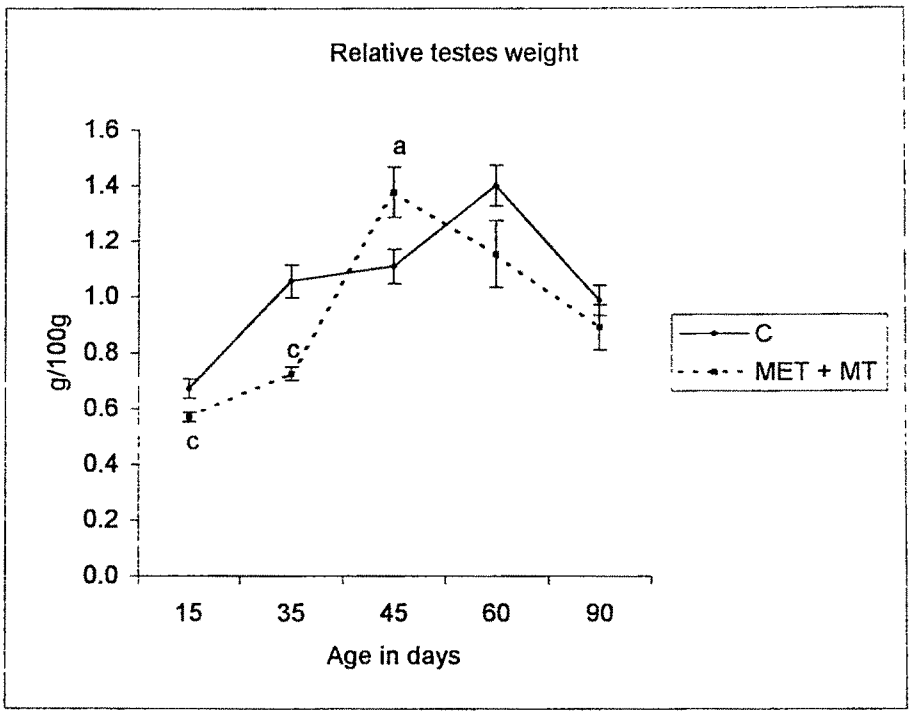
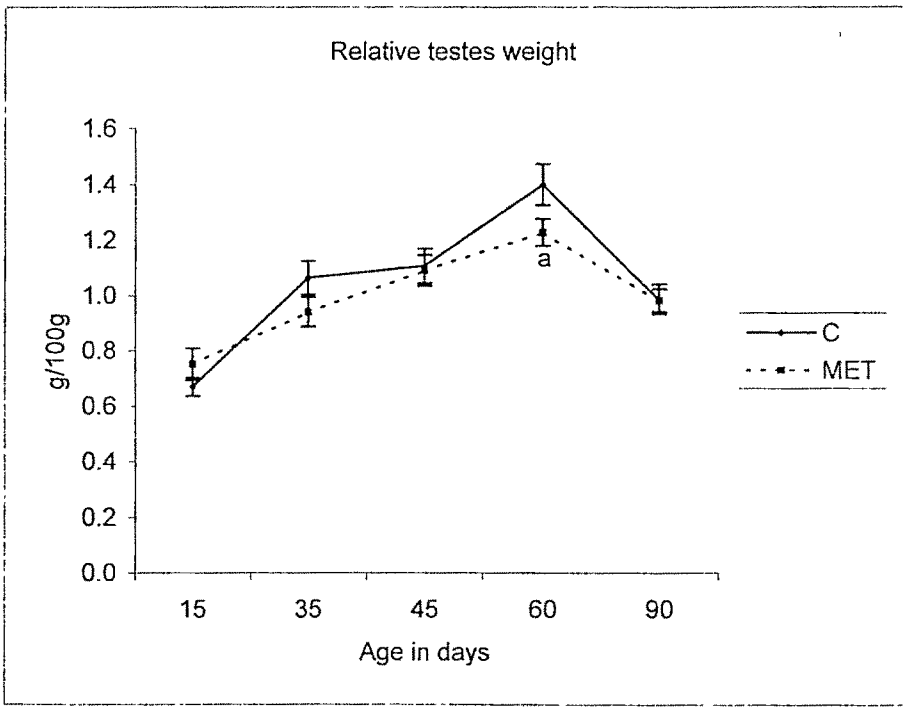


Fig.2: Chronological alterations in relative weight (g/100 g) of testes in Control and Metyrapone + Melatonin treated rats

C - Control, **MET+MT** - Metyrapone+ Melatonin treated rats

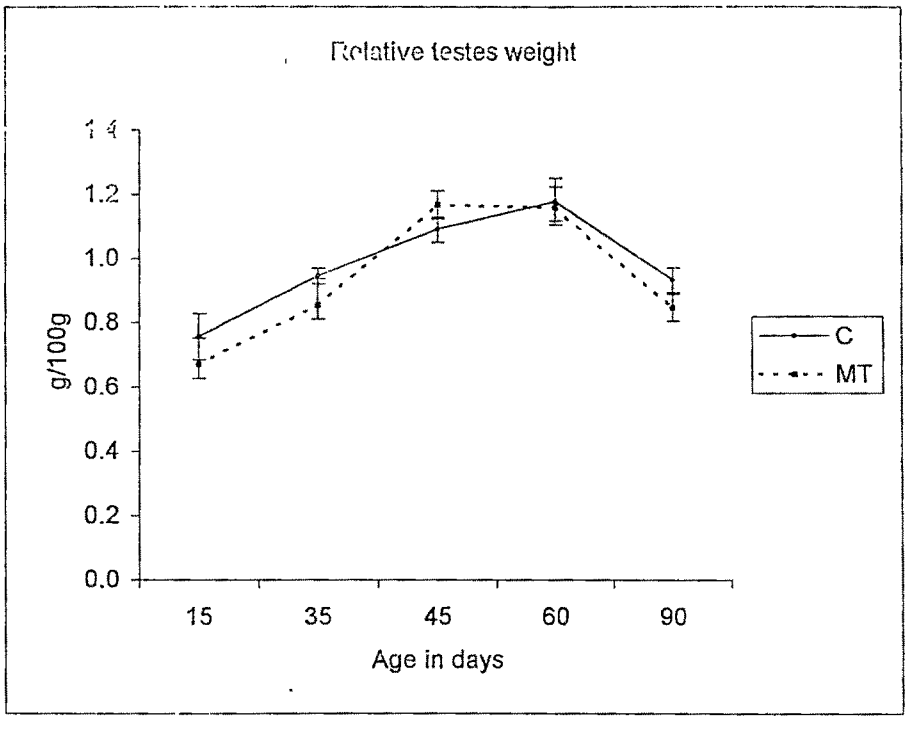
Values expressed as Mean ± SEM of six animals

a $p < 0.05$, b $p < 0.005$, c $p < 0.0005$



. Chronological alterations in relative weight (g/100 g) of testes in Control and Metyrapone treated rats

C - Control, **MET** - Metyrapone, Values expressed as Mean ± SEM of six animals, [a] p< 0 05, [b] p< 0 005, [c] p< 0.0005



Relative testes weight (g/100g) in Control and Melatonin treated rats

C – Control, MT – Melatonin treated

Values expressed as Mean ± SEM of six animals [a] $p< 0.05$, [b] $p< 0.005$, [c] $p< 0.0005$

Table 2: Per day Body and Testes Growth Rate (g/day) in Control and Metyrapone + Melatonin treated rats.

Treatment	Per Day Body Growth Rate					Per Day Testes Growth Rate				
	Age in Days					Age in Days				
	0-15	15-35	35-45	45-60	60-90	0-15	15-35	35-45	45-60	60-90
C	1.945	2.36	4.05	4 988	5.452	0.014	0.032	0 049	0.084	0.033
MET + MT	1.535	2.837	6.866	5.656	3.483	0.011	0.025	0.094	0.061	0.029
* C	2.117	2.409	3.165	5.100	4.294	0.19	0.0261	0.148	0.052	0.102
MT	1.663	3.942	4.200	5.422	5.500	0.014	0.035	0.084	0.062	0.022
* C	1.944	2.36	1.05	4.988	5.42	0.014	0.032	0.048	0.083	0.032
MET	1.633	3.45	7.5	5.55	3.194	0.011	0.04	0.105	0.084	0.010

C – Control, **MET + MT** - Metyrapone+ Evening Melatonin injection, **MET** – Metyrapone, **MT** - Evening Melatonin injection

* Values recalled from Chapters 1and 6

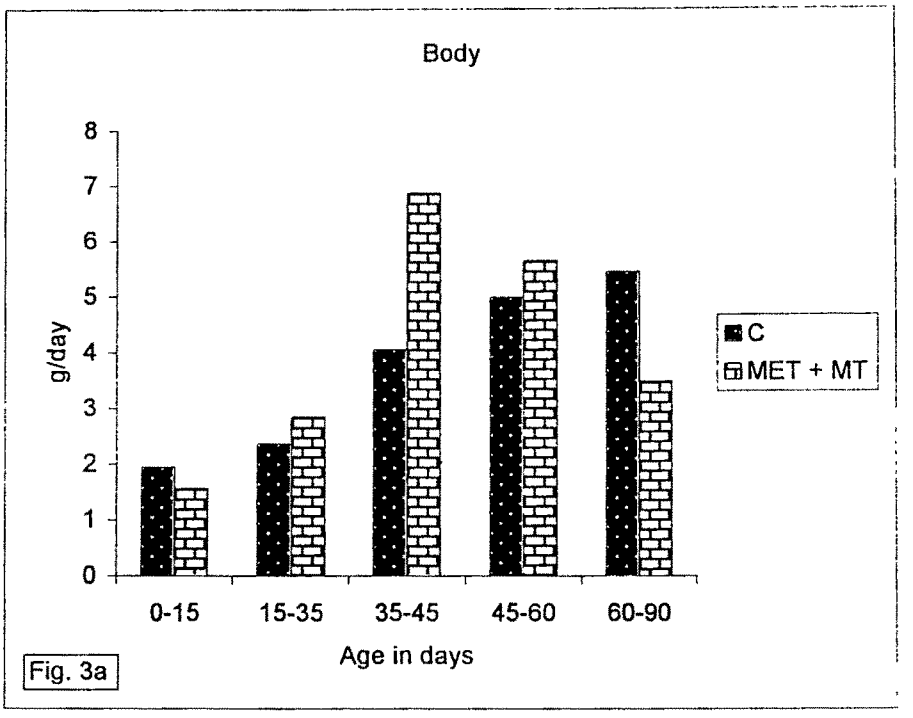


Fig. 3a

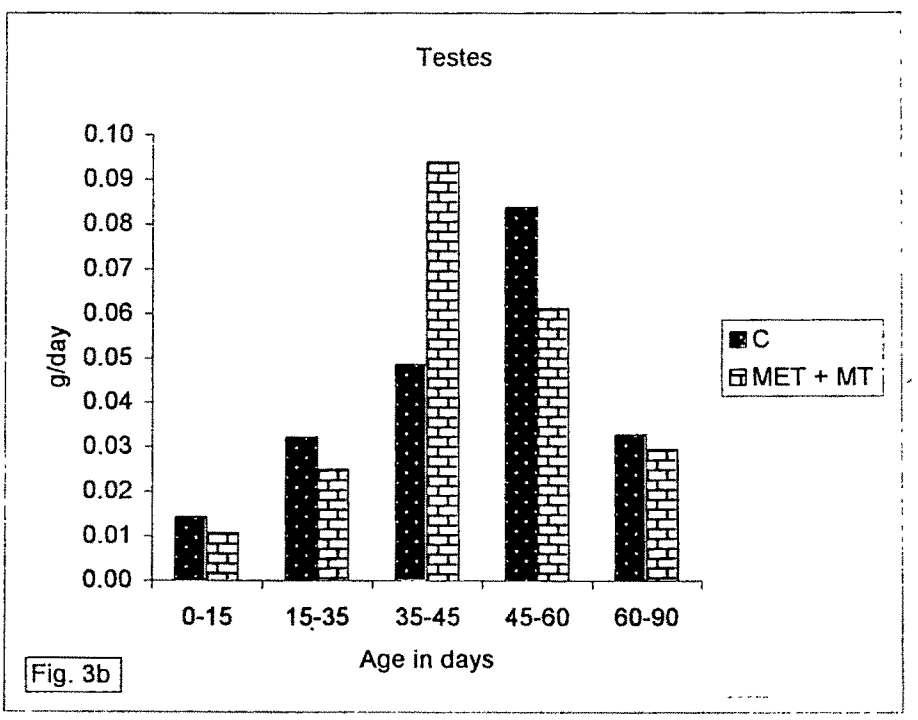
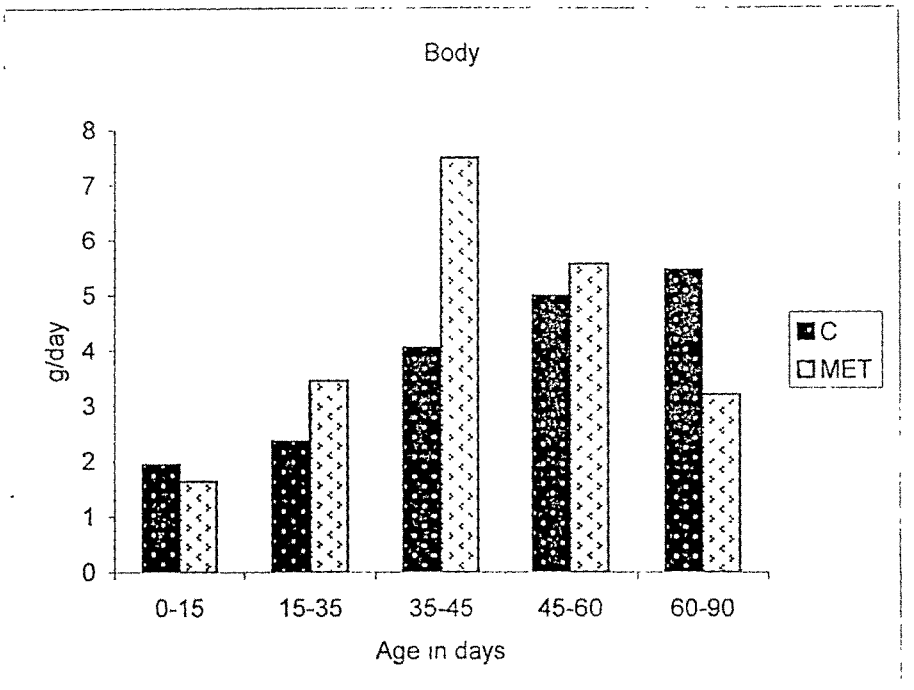
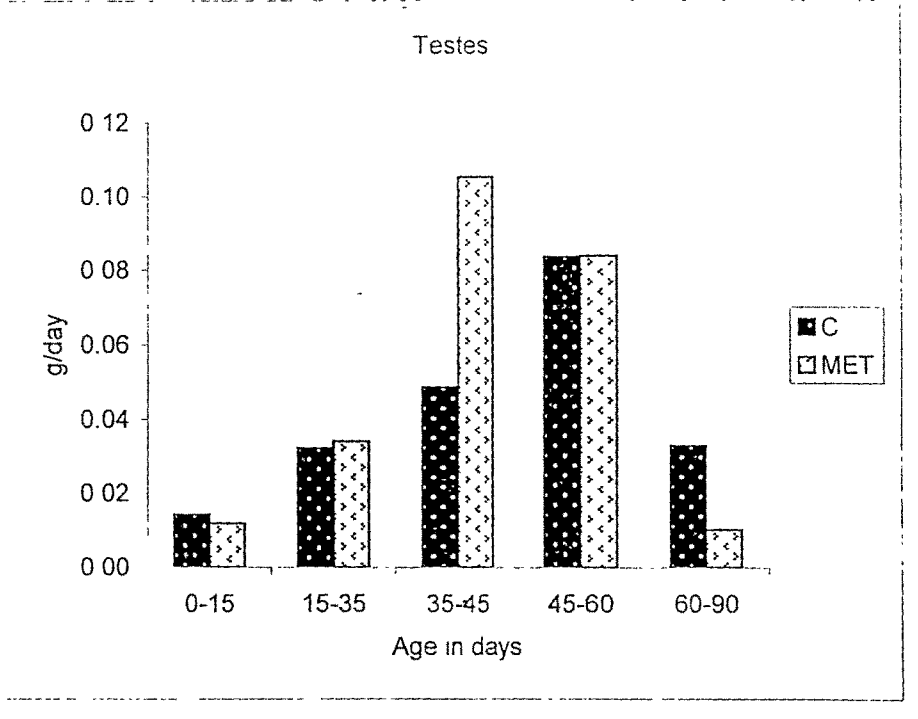


Fig. 3b

Fig. 3a and 3b: Per day Body and Testes Growth rate (g/day) in Control and Metyrapone + Melatonin treated rats

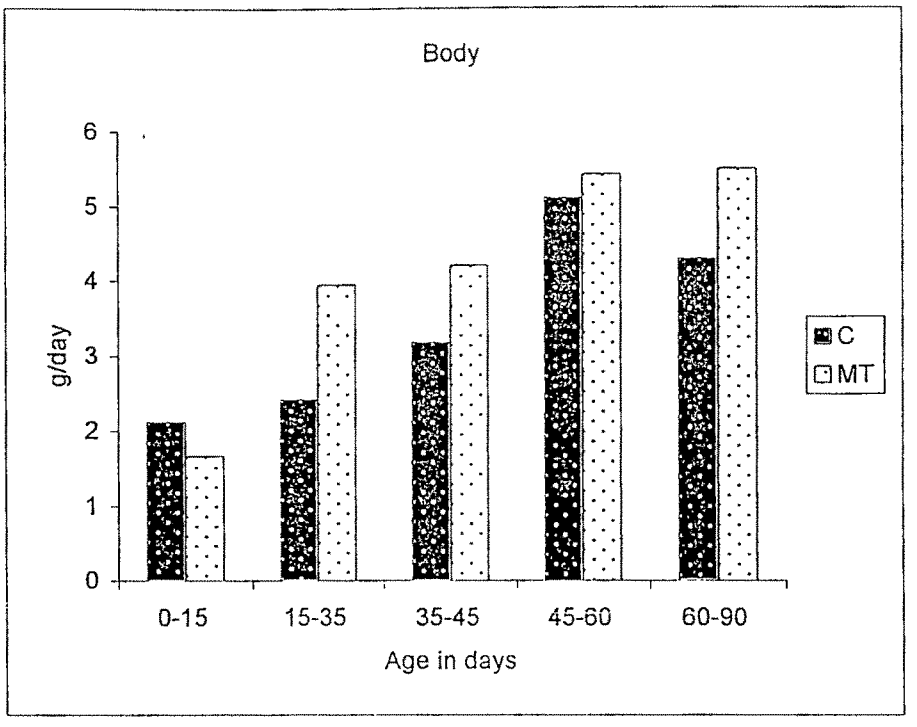
C - Control, **MET+MT** - Metyrapone+ Melatonin treated rats

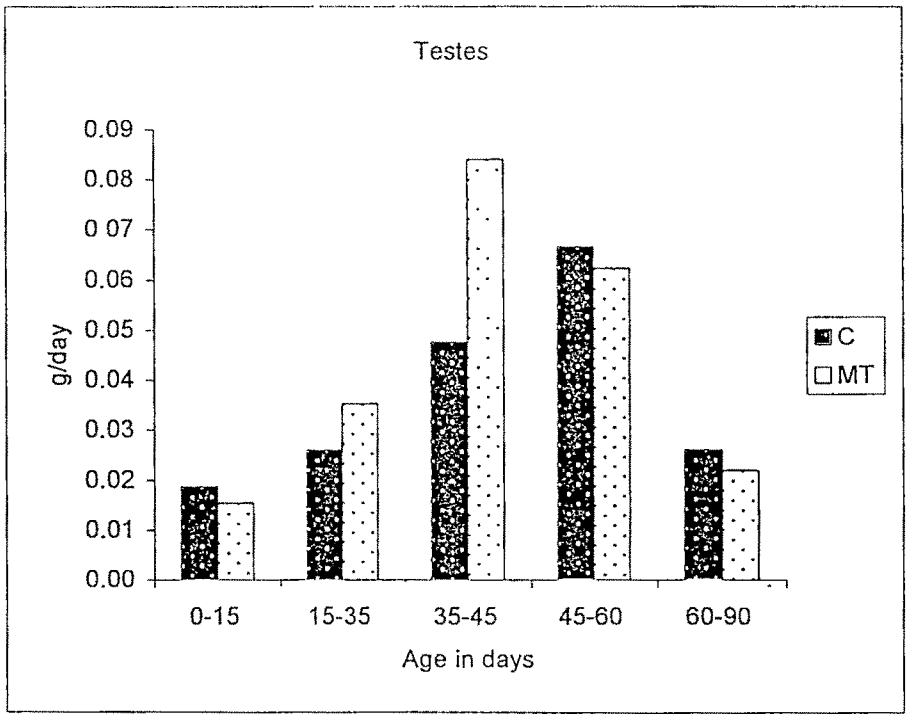




Per day Body and Testes Growth rate (g/day) in Control and Metyrapone treated rats

C - Control, **MET** - Metyrapone





Per day Body and Testes growht rate (g/day) in Control and Melatonin treated rats

Table 3: Histometric enumerations in seminiferous tubules in Control and Metyrapone + Melatonin treated rats at 90 days

Treatment	T_V in cc	S_D in cm	GE in cm	S_V in cc	S_L in cm	bm in cm^2	$SC_N \times 10^6$	$TGC_T \times 10^6$	$AGC_T \times 10^6$	$TGC_M \times 10^6$	$AGC_M \times 10^6$	% Loss
C	1.503 ±0.030	0.0279 ±0.0006	0.0074 ±0.0003	1.427 ±0.050	2321.03 ±94.200	204.045 ±5.230	32.49 ±1.800	311 ±6.300	280.84 ±5.600	13.39 ±0.260	12 1 ±0.150	10.00 ±0.0002
MET+MT	1.389[b] ±0.010	0.0320[c] ±0.0006	0.0098[c] ±0.0003	1.319[a] ±0.035	1616.8[c] ±25.300	163.73[c] ±1.900	22.635[c] ±1.600	311.00 ±2.900	258.85[b] ±4.200	19.24[c] ±0.350	16.000[c] ±0.048	16.830[c] ±0.045
* MT	1.541 ±0.070	0.033[c] ±0.001	0.0098 ±0.002	1.448 ±0.060	1725.2[c] ±45.30	177.20[c] ±4.690	24.15[b] ±1.600	340.00[b] ±5.600	279.45 ±2.800	19.70[c] ±0.350	16.20[c] ±0.210	19.00[c] ±0.200
* MET	1.589 ±0.090	0.0290 ±0.0010	0.0088 ±0.0001	1.525 ±0.070	2323.66 ±56.900	211.040 ±4 500	32.531 ±1.900	361.00[c] ±4.600	232.27[c] ±3.800	14.430 ±0.659	9.990 ±1.260	30.760[c] ±0.150

C – Control, **MET+MT** – Metyrapone + Melatonin evening injection treated rats

Values expressed as Mean ± SEM of minimum fifteen observations. [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$

* Values recalled from Chapters 1 and 6

T_V - Volume of Testis, **S_D** - Seminiferous tubule diameter, **GE** - Germinal epithelial thickness, **S_V** - Volume of Seminiferous tubule, **S_L** - Length of seminiferous tubule, **bm** - basement membrane area of the seminiferous tubule, **SC_N** - Total Sertoli cell number in testis, **TGC_T** - Theoretical germ cell number per testis, **AGC_T** - Actual germ cell number per testis, **TGC_M** - Theoretical germ cell number per meter of seminiferous tubule, **AGC_M** - Actual germ cell number per meter of seminiferous tubule.

prominent. The tubular diameter and germinal epithelial thickness were lesser than the controls at 60 days but, at 90 days, both were significantly greater in MET+MT animals. There was significant decrease in testicular and tubular volumes, tubular length and total basement area and total Sertoli cell number per testis. The total theoretical germ cell number per testis was however normal and identical to that of age matched controls (Table 3). However, in terms of unit length of the seminiferous tubule, the germ cell number was significantly higher and the actual number of germ cells based on counts made from the sections, revealed a significantly higher number. The difference between the two counts taken as the degree of degeneration/germ cell loss was also significantly greater than in the controls. The data on tubular length, total basement area, Sertoli cell count and germ cell number and degree of degeneration were all reversed compared to MET alone animals and similar to melatonin alone animals (Plates I, II, Xa and Xb).

Serum Hormone Profile:

Corticosterone:

During the treatment period, corticosterone level was significantly lower in the experimental animals. Like the control animals, the experimental animals also showed a continuous increase in the hormone level between 15-90 days. However, the significant and steep increase, which occurs between

45 and 60 days, was not shown by MET+MT animals with the result, the adult levels of corticosterone were significantly lower compared to controls (Table 4; Figure 4). The levels at 35 and 45 days were slightly more in experimentals. These changes in the serum corticosterone titre were quite different from those shown by either MT or MET animals (Table 4).

TSH, T_4 and T_3:

The serum TSH levels in the experimental animals were in general significantly higher at all ages as compared to the age-matched controls with maximal level at 90 days (Table 5; Figure 6). The serum T_4 and T_3 levels were significantly higher in the MET+MT animals during the treatment period (15 days) and the immediate post-treatment period (35 days). Subsequently, whereas the serum T_4 level was comparable to the controls at all ages, that of T_3 was found to be subnormal in the adult stage (60-90 days) (Table 5; Figures 7a & 7b). The overall changes in the levels of TSH, T_4 and T_3 shown by MET+MT animals were again quite distinct than that shown by MT or MET animals (Table 5).

LH and Testosterone:

The serum LH level increased continuously from 15-90 days in both controls and experimental animals. However, the experimentals showed significantly lower levels at all ages with the difference becoming more pronounced with age (Table 4; Figure 5a). In contrast, serum testosterone levels which also show similar continuous increase in controls was not shown

Table 4: Serum Corticosterone, LH and T levels (ng/ml) in Control and Metyrapone + Melatonin treated rats.

Treatment	Corticosterone					LH					T				
	Age in days					Age in days					Age in days				
	15	35	45	60	90	15	35	45	60	90	15	35	45	60	90
C	5 825 ±0.085	8.000 ±0.618	10.150 ±0.155	48.300 ±0.705	45.00 ±1.699	9.390 ±0.132	16.450 ±0.634	21.75 ±0.854	48.125 ±1.235	53.250 ±1.031	0.235 ±0 019	0.550 ±0.166	2.235 ±0.278	2.625 ±0.217	4.375 ±0.620
MET + MT	3 0^{c} ±0 084	10.2^{b} ±0.169	15.0^{c} ±0.283	18.0^{c} ±0.564	15.8^{c} ±0.489	7.13^{c} ±0.086	9.15^{c} ±0.10	10.3^{c} ±0.11	11.4^{c} ±0.09	12.92^{c} ±0.13	0.73^{c} ±0.02	1.4^{b} ±0.03	1.56^{a} ±0.022	1.20^{c} ±0.026	1.60^{b} ±0.08
* MT	12 40^{c} ±1.106	24.70^{c} ±2.075	13.40^{a} ±1.028	44.01 ±4.732	43.01^{c} ±4.856	8.761 ±1.009	12.17^{b} ±1.024	16.39^{b} ±1.008	17.77^{c} ±1.025	20.99^{c} ±2.025	0.221 ±0.016	1.501^{c} ±0.048	1.503^{a} ±0.054	1.300^{c} ±0.058	2.002^{b} ±0.088
* MET	2 7 ±0.015	23.0 ±0.654	20.0 ±0.850	24.8 ±0.746	39.0 ±0.156	11.4^{a} 0.967	28.56^{c} ±1.24	71.25^{c} ±2.876	86.35^{c} ±2.678	56.32 ±2.794	0.485^{c} ±0.028	1.954^{c} ±0.042	2.150 ±0.016	2.569 ±0.026	1.48^{b} ±0.034

C – Control, **MET + MT** - Metyrapone+ Evening Melatonin injection, **MET** – Metyrapone,
MT - Evening Melatonin injection

Values expressed as Mean ± SEM of four samples. ^{a} $p < 0.05$, ^{b} $p < 0.005$, ^{c} $p < 0.0005$

* Values recalled from Chapters 1 and 6

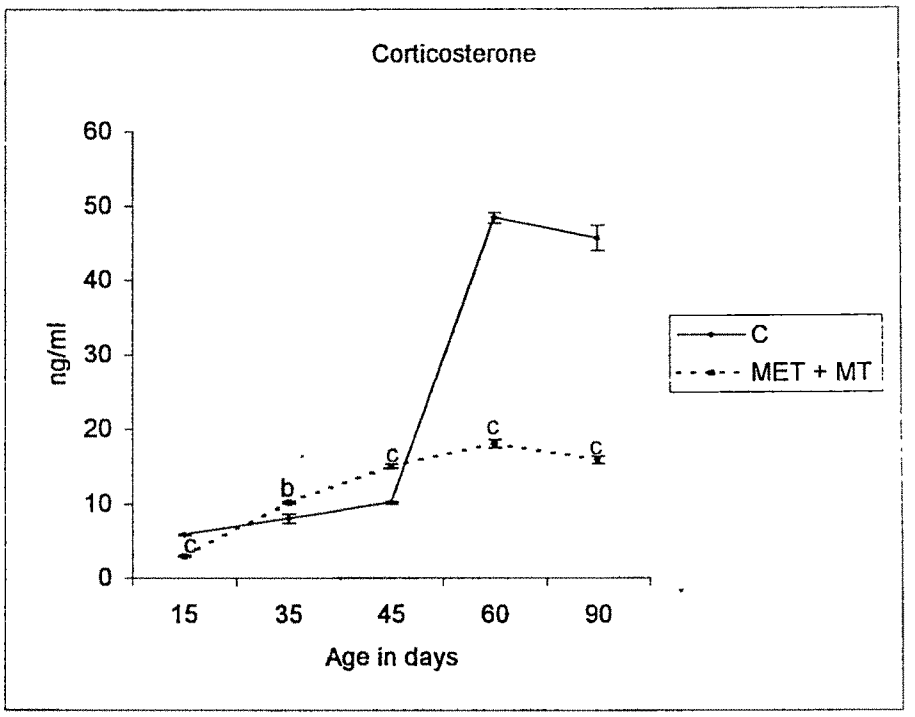
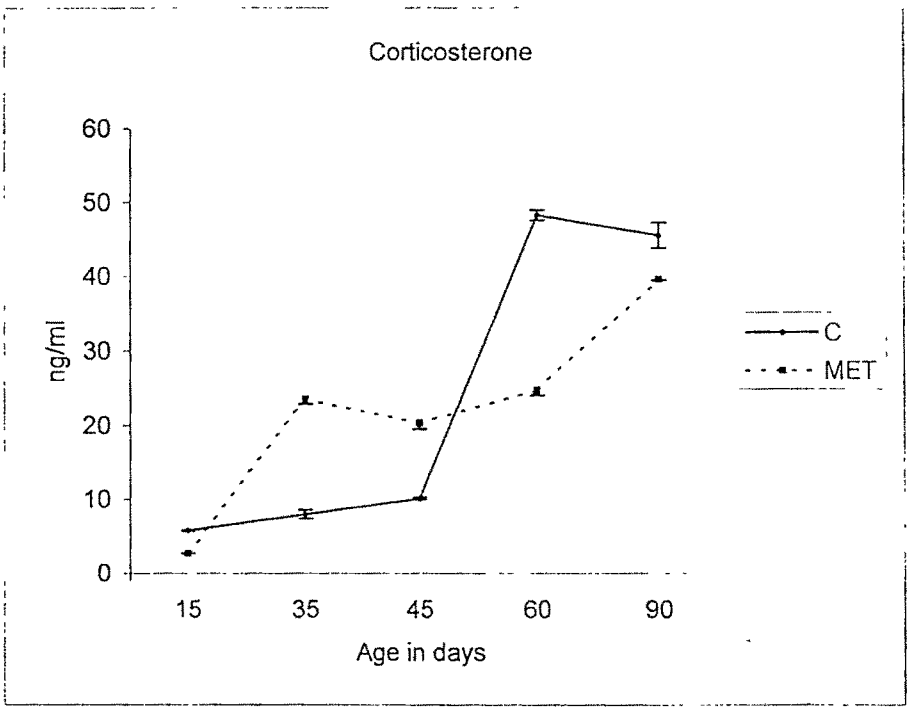


Fig.4: Serum Corticosterone level (ng/ml) in Control and Metyrapone + Melatonin treated rats

C - Control, **MET+MT** - Metyrapone+ Melatonin treated rats

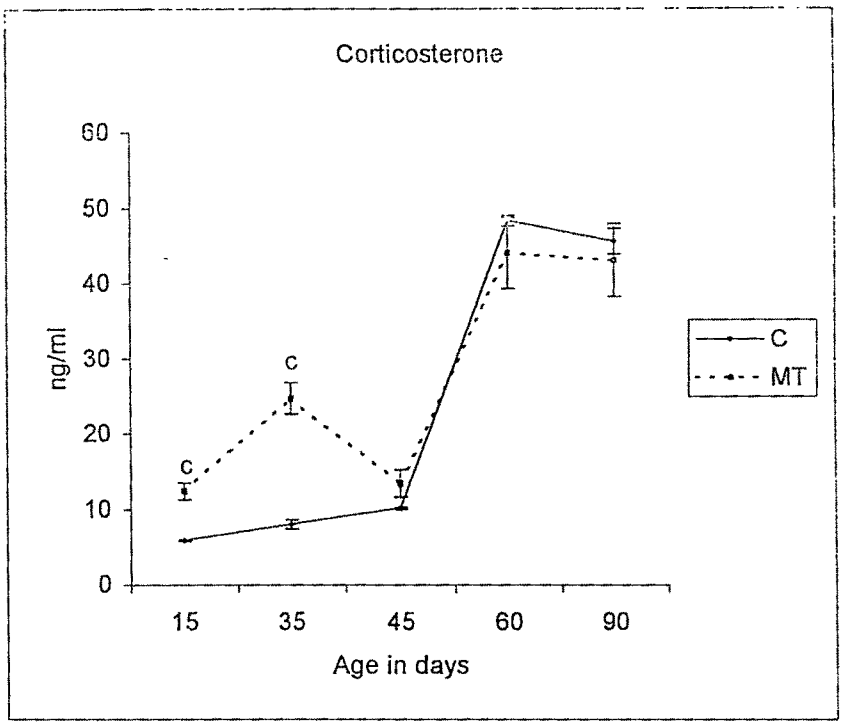
Values expressed as Mean ± SEM of four samples

a $p < 0.05$, b $p < 0.005$, c $p < 0.0005$



Serum Corticosterone level (ng/ml) in Control and Metyrapone treated rats

C - Control, **MET** - Metyrapone, Values expressed as Mean ± SEM of four samples, [a] $p < 0.05$, [b] $p < 0\ 005$, [c] $p < 0\ 0005$



Serum Corticosterone level (ng/ml) in Control and Melatonin treated rats

C – Control, **MT** – Melatonin treated

Values expressed as Mean ± SEM of four samples

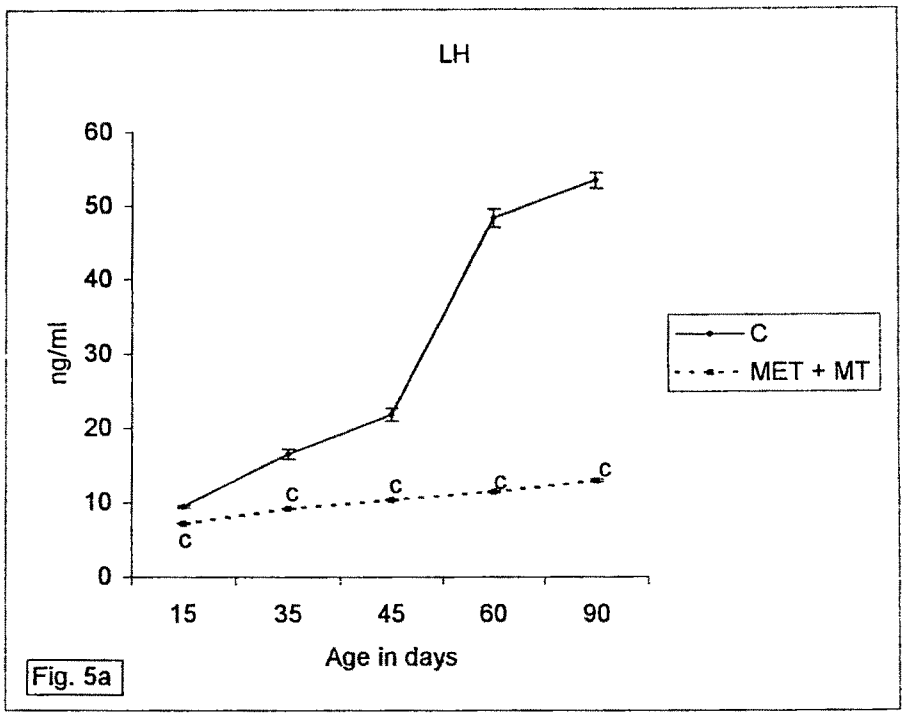
a $p < 0.05$, b $p < 0.005$, c $p < 0.0005$

Table 5: Serum TSH, T_4 and T_3 levels (ng/ml) in Control and Metyrapone + Melatonin treated rats

Treatment	TSH					T_4					T_3				
	Age in days					Age in days					Age in days				
	15	35	45	60	90	15	35	45	60	90	15	35	45	60	90
C	3 175 ±0 165	6.600 ±0.129	6.873 ±0.111	7.495 ±0.143	5.440 ±0.066	0.31 ±0.013	0.583 ±0.085	1.170 ±0.061	2.568 ±0.024	2.368 ±0.225	0.215 ±0.051	0.450 ±0.011	0.303 ±0.107	0.603 ±0.084	0.653 ±0.053
MET+ MT	5 3^{c} ±0.086	9.7^{c} ±0.099	4.4^{c} ±0.073	9.8^{c} ±0.096	14.5^{c} ±0.08	1.4^{c} ±0.024	2.8^{c} ±0.036	1.01^{a} ±0.021	2.9^{c} ±0.030	2.05^{c} ±0.031	0.53^{c} ±0.012	0.82^{c} ±0.011	0.37^{a} ±0.014	0.39^{c} ±0.011	0.28^{c} 0.026
* MT	14.01^{c} ±0.104	26.01^{c} ±1.577	9.050^{c} ±0.804	7.804^{c} ±804	9.001^{c} ±0.365	1.282^{c} ±0.084	1.700^{c} ±0.053	2.805^{c} ±0.015	2.212^{b} ±0.068	1.742^{c} ±0.018	0.400 ±0.026	0.481 ±0.056	0.800^{c} ±0.074	1.051^{c} ±0.084	0.910^{c} ±0.095
* MET	2 254^{b} ±0.134	6.980 ±0.243	7.250 ±0.269	7.840 ±0.341	9.640^{c} ±0.398	0.46^{b} ±0.024	1.94^{c} ±0.024	3.0^{c} ±0.032	1.73^{c} ±0.032	1.74^{b} ±0.021	0.125 ±0.025	0.205^{c} ±0.021	0.285 ±0.012	0.264^{b} ±0.016	0.367^{b} ±0.019

C – Control, **MET+ MT** – Metyrapone + Evening Melatonin injection, **MET** – Metyrapone, **MT** - Evening Melatonin injection

Values expressed as Mean ± SEM of four Samples. ^{a} $p < 0.05$, ^{b} $p < 0.005$, ^{c} $p < 0.0005$



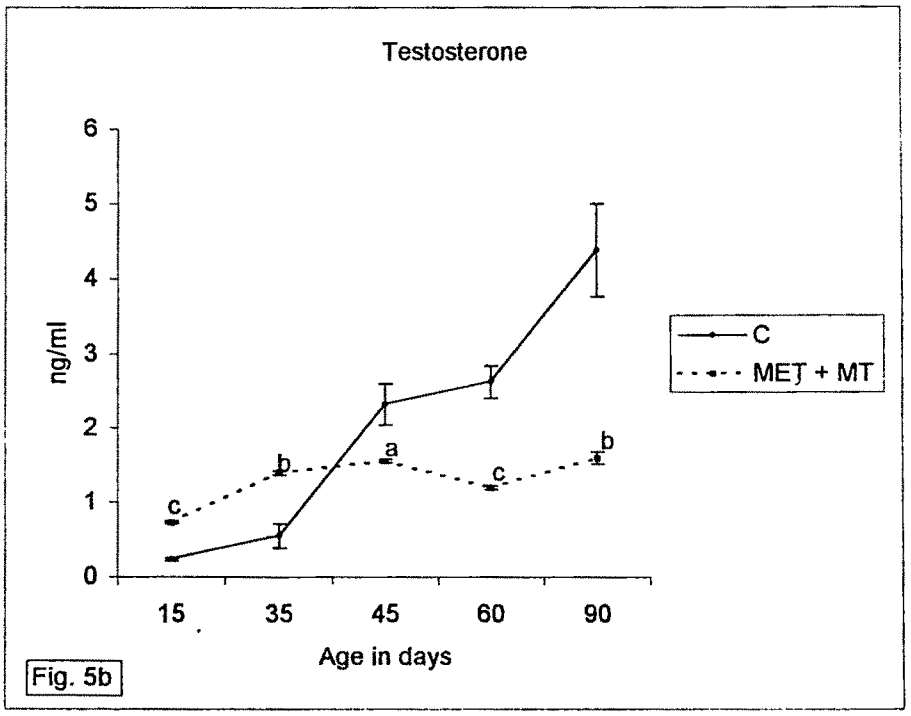
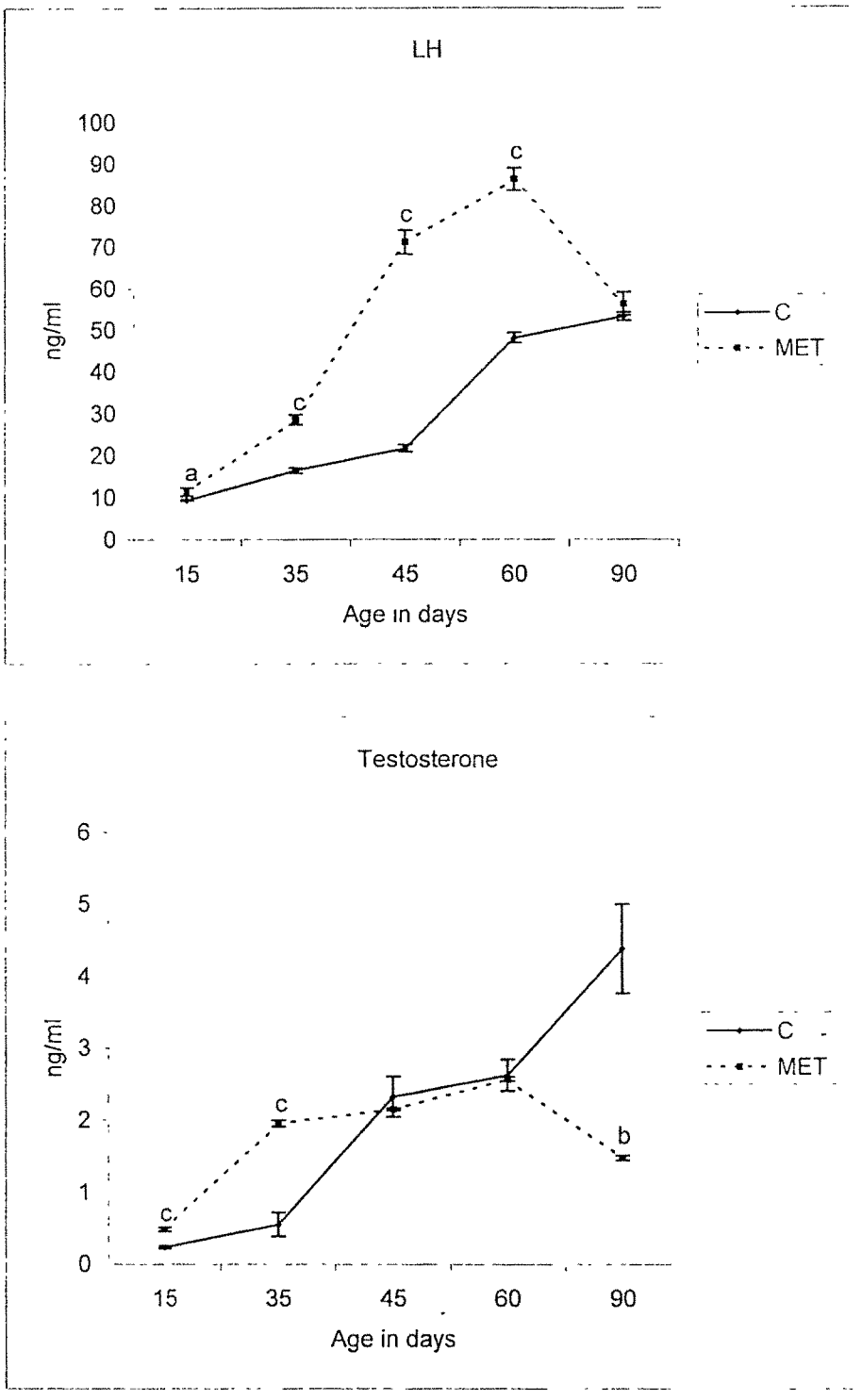


Fig.5a and 5b: Serum LH and T levels (ng/ml) in Control and Metyrapone + Melatonin treated rats

C - Control, **MET+MT** - Metyrapone+ Melatonin treated rats

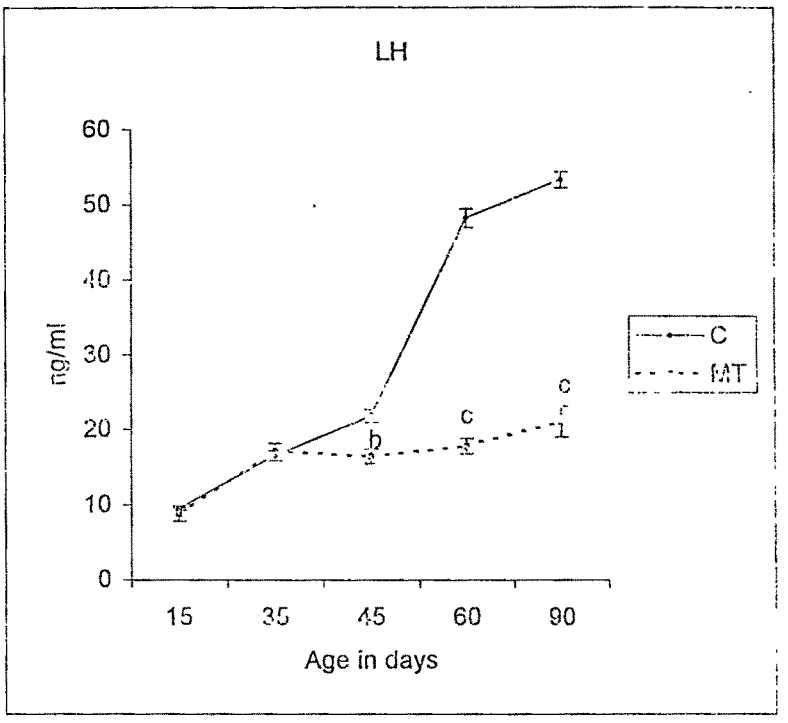
Values expressed as Mean ± SEM of four samples

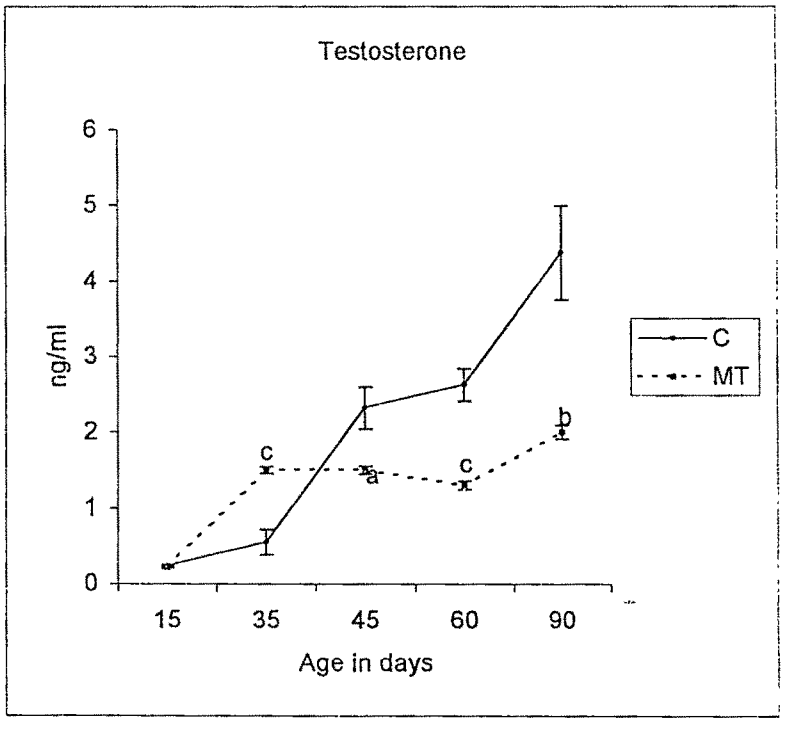
a $p < 0.05$, **b** $p \leq 0.005$, **c** $p < 0.0005$



Serum LH and T levels (ng/ml) in Control and Metyrapone treated rats

C - Control, MET - Metyrapone, Values expressed as Mean ± SEM of four samples, [a] p< 0 05, [b] p< 0.005, [c] p< 0.0005





Serum LH and T levels (ng/ml) in Control and Melatonin treated rats

C – Control, **MT** – Melatonin treated

Values expressed as Mean ± SEM of four samples

[a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$

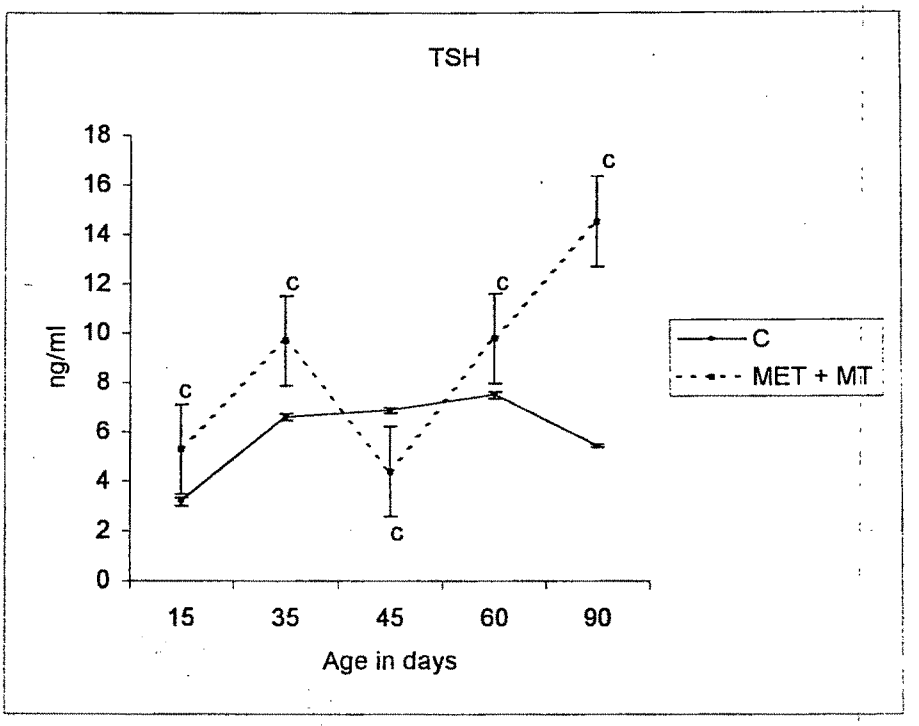
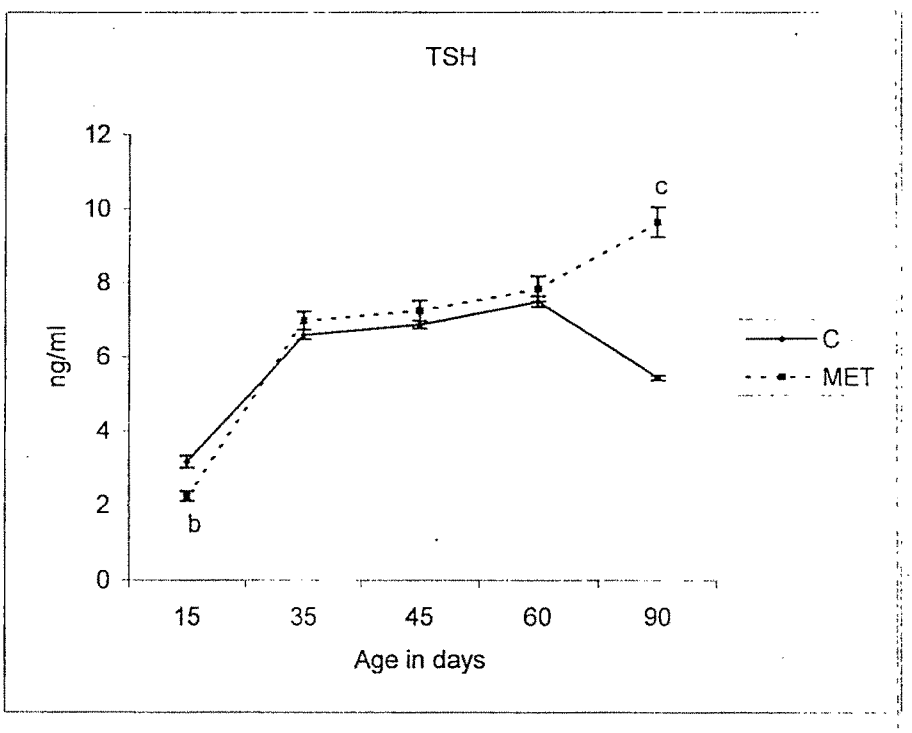


Fig.6: Serum TSH level (ng/ml) in Control and Metyrapone + Melatonin treated rats

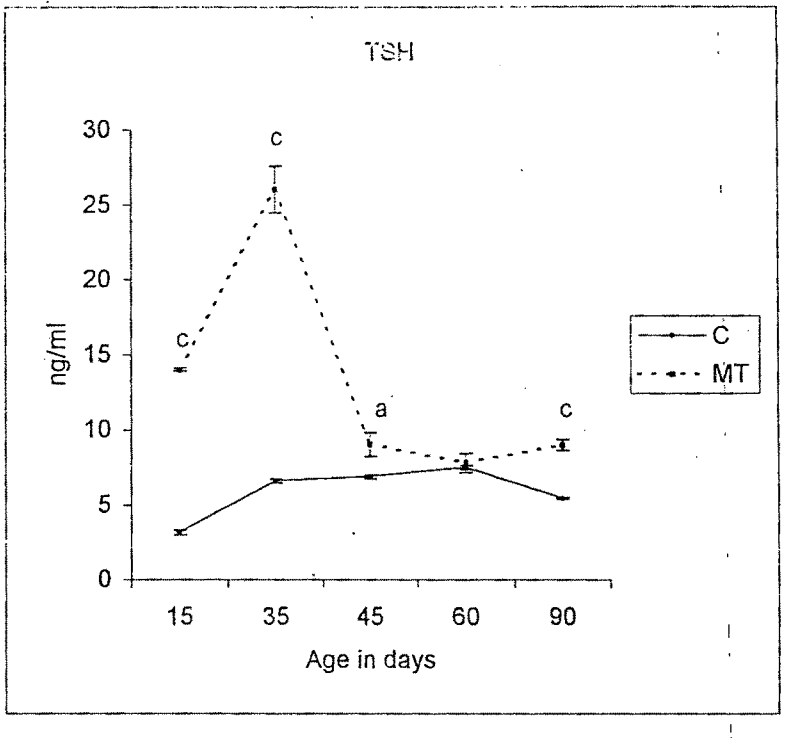
C - Control, **MET+MT** - Metyrapone+ Melatonin treated rats

Values expressed as Mean ± SEM of four samples

a $p < 0.05$, b $p < 0.005$, c $p < 0.0005$



Serum TSH level (ng/ml) in Control and Metyrapone treated rats
C - Control, **MET** - Metyrapone, Values expressed as Mean ± SEM of four samples, [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$



Serum TSH level (ng/ml) in Control and Melatonin treated ra

C – Control, **MT** – Melatonin treated

Values expressed as Mean ± SEM of four samples

[a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$

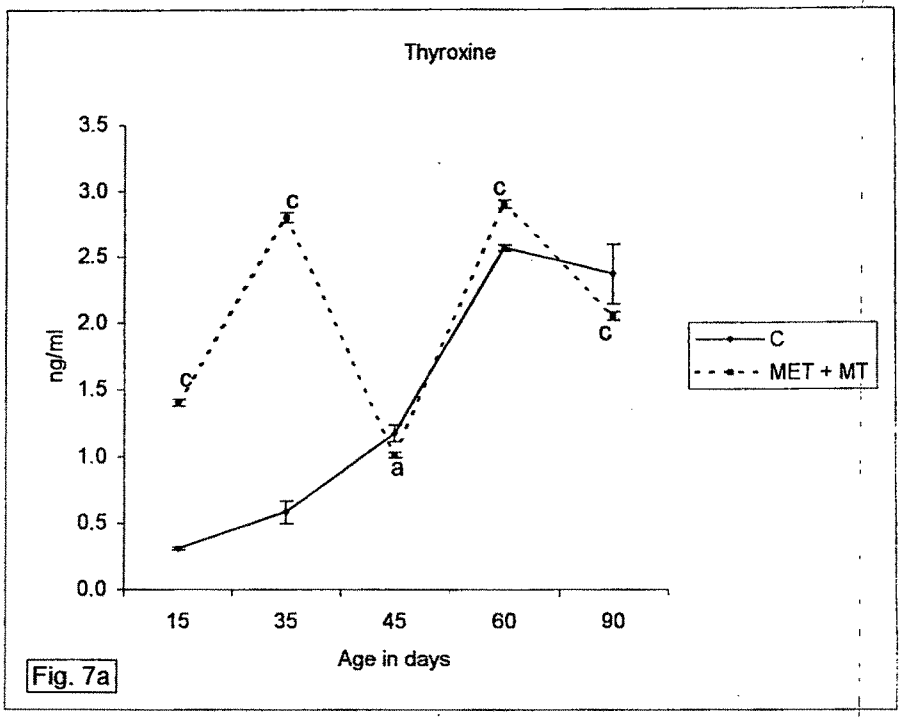


Fig. 7a

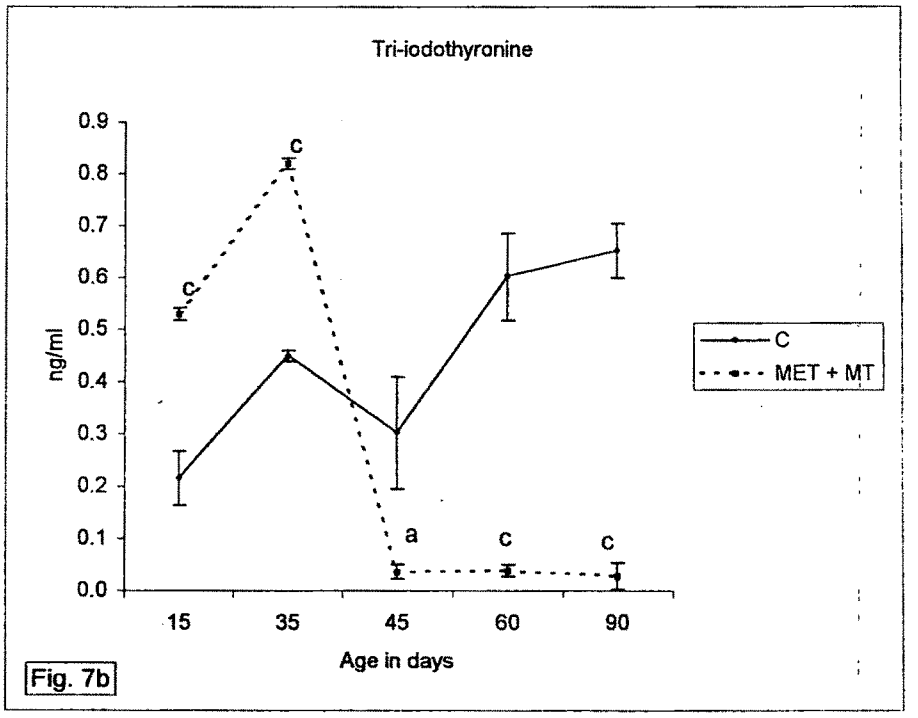


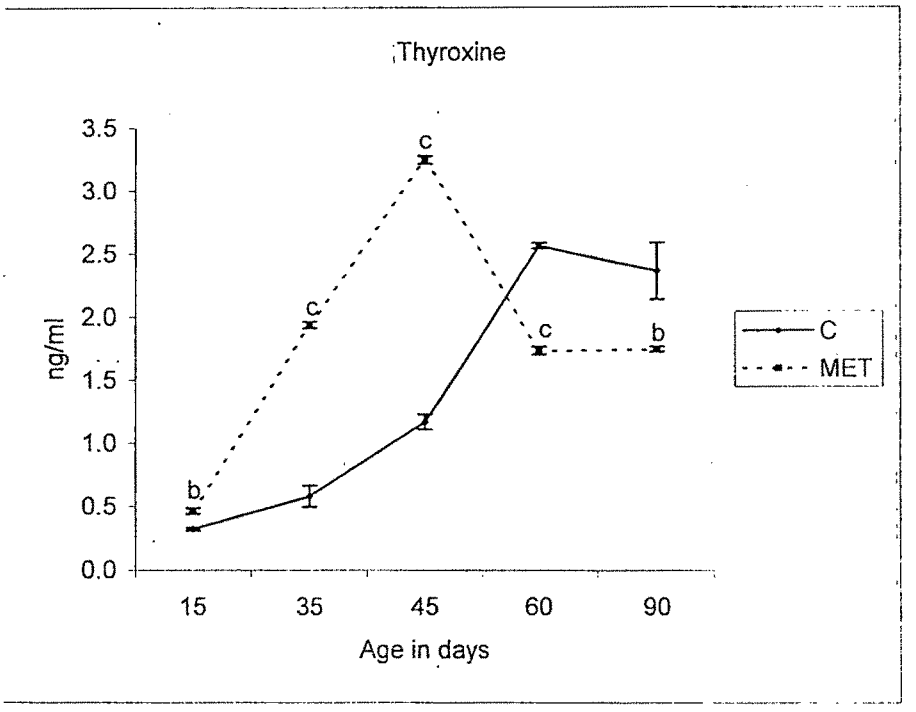
Fig. 7b

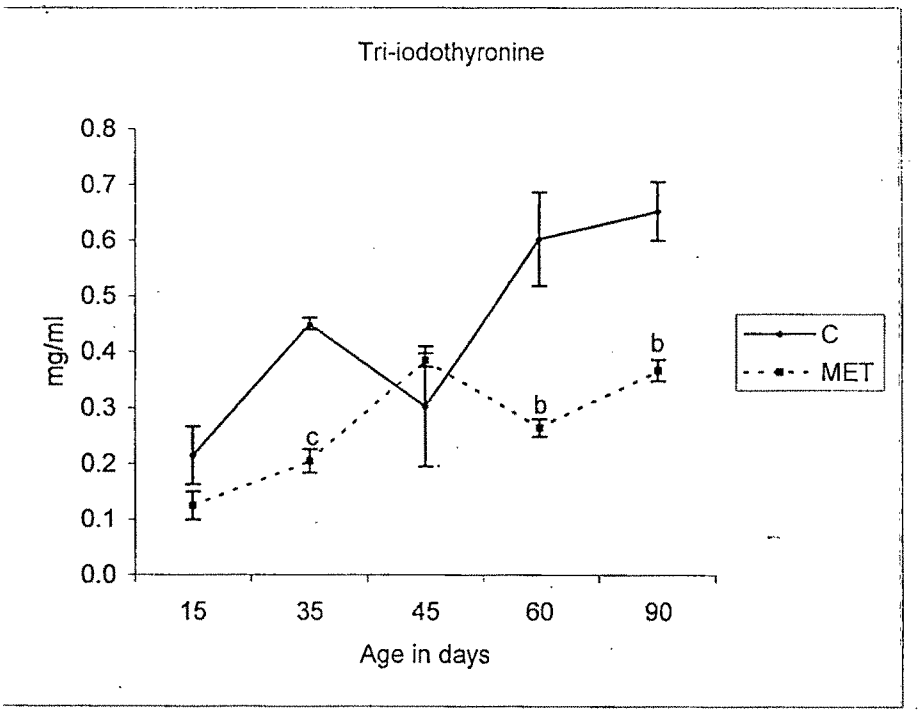
Fig.7a and 7b: Serum T_4 and T_3 levels (ng/ml) in Control and Metyrapone + Melatonin treated rats

C - Control, **MET+MT** - Metyrapone+ Melatonin treated rats

Values expressed as Mean ± SEM of four samples

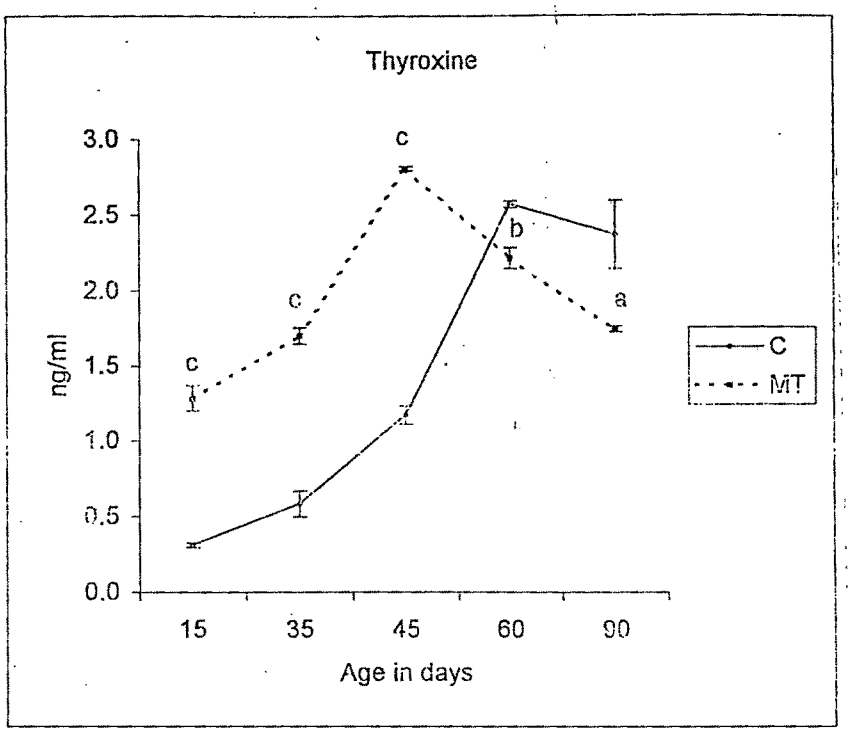
a $p< 0.05$, b $p< 0.005$, c $p< 0.0005$





Serum T_4 and T_3 levels (ng/ml) in Control and Metyrapone treated rats

C - Control, **MET** - Metyrapone, Values expressed as Mean ± SEM of four samples, [a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$



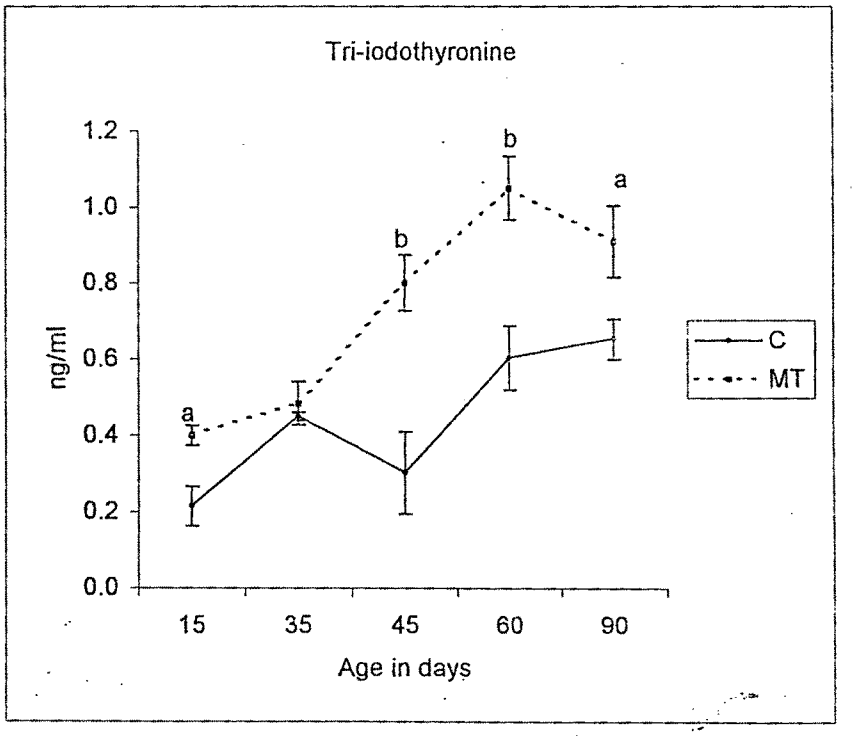


Fig.7a and 7b: Serum T_4 and T_3 levels (ng/ml) in Control and Melatonin treated rats.

C – Control, **MT** – Melatonin treated

Values expressed as Mean ± SEM of four samples

[a] $p < 0.05$, [b] $p < 0.005$, [c] $p < 0.0005$

by the experimental animals. Though the testosterone level was significantly higher in MET+MT animals at 15 and 35 days, there was no further increase after 35 days and remained steady thereafter at that level (Table 4; Figure 5b). The changes in the levels of LH and testosterone in MET+MT animals were not identical to that shown by MET or MT treated animals (Table 4).

DISCUSSION:

The data on body and testes weight and growth rates reveal hastened growth kinetics in the experimental animals with peak growth rate for both body and testes occurring between 35-45 days as against 60-90 days for body and 45-60 days for testes weights in the controls (Table 2; Figures 3a & 3b). Whereas the pattern of body growth rate mimics that of MET treated animals, that of testes and the relative weight of testes resemble those seen in the melatonin treated animals (Table 1 and 2). The hastened growth of body and testes in the prepubertal period seems to have a relation with the increased corticosterone levels at 35 and 45 days, as has been inferred in the previous work (Chapter 6). The influence of melatonin on testes weight in the adult is further potentiated by neonatal hypocorticalism, as the percentage difference in the relative weight of the testes in MET+MT animals at 90 days, compared to the age matched controls, is higher than the difference between melatonin and control animals.

The effect on the testes due to neonatal MET+MT treatment is well denoted by the decreased testicular and tubular volumes, reduced tubular length, basement membrane area and Sertoli cell number. These changes are not only similar to those recorded for MT treated animals rather than MET treated animals, but also of greater degree than those recorded for MT animals. Obviously, the influence of melatonin on the growth kinetics of testes constituents is more potentiated with simultaneous hypocorticalism. Similarity with melatonin treatment is further emphasized by the recorded theoretical total number as well as the actual number of germ cells per unit length of tubule (Table 3). The actual number of germ cells per unit length of tubule was found to be significantly lower in MET animals (Table 3). The significantly higher number of germ cells in the present experimental animals which appears similar to that recorded for MT animals is suggestive of the nullifying influence of melatonin over that of hypocorticalism. The difference between the theoretically possible germ cells and the actual number of germ cells, which is taken as the loss due to degeneration/apoptosis, is also significantly lower in MET+MT animals compared to MET animals. This once again alludes to the protective action of melatonin in countering hypocorticalism induced apoptotic loss of germ cells. The percentage loss of germ cells recorded in the present study is very much comparable to those recorded for hypermelatonemic animals, though slightly more. Interestingly, the increased degenerative changes that could be seen in the histological

picture of testis of melatonin animals are not observable in the MET+MT animals, thereby suggesting a nullifying influence of hypocorticalism on the melatonin effect in this regard. Taken to a logical conclusion, this suggests the need for an optimum corticosterone *milieu* for the expression of the melatonin effect on degenerative changes of germ cells. The possible mechanisms of melatonin induced germ cell degeneration have already been discussed in a previous work (Chapter 1). In brief, it can be said that the melatonin-induced alterations in the adhesional properties between Sertoli cells and germ cells can find greater expression in conjunction with higher corticosterone level and vice versa under a simultaneous corticosterone deficit.

With regard to the influence of neonatal MET+MT treatment on adult hormonal profiles, it is seen that the alterations are unique and quite distinct from those recorded either in MT or MET animals. This would suggest synergistic as well as antagonistic interactions between hypermelatonemia and hypocorticalism. The corticosterone profile suggests a permanent lowering of the central set point of hypothalamo-hypophyseal-adrenal (HHA) axis due to a combination of melatonin excess and corticosterone deficit during the neonatal period. This is quite in distinction from that seen in the MT or MET treatment alone (Table 4). The hypothalamo-hypophyseal-thyroid (HHT) axis also seems to be affected in a very distinct way. At the pituitary level, neonatal MET+MT seems to either increase the central set point

resulting in increased thyrotrophin releasing hormone (TRH) output and/or an increased sensitivity of thyrotropes as seen by the significantly high level of TSH throughout. At the thyroid level, not only is the sensitivity towards TSH significantly reduced but, also shows a differential effect in terms of the release of T_4 and T_3. Whereas the T_4 levels were significantly higher during the treatment and immediate post-treatment periods (15-35 days) and, in the control range thereafter, the T_3 levels were significantly and consistently lower in the adult condition (60 and 90 days) though significantly higher during the treatment and post-treatment periods. Clearly, there are intricate interactions of hypermelatonemia and hypocorticalism in the neonatal stage on the modulation of HHT axis. The hypothalamo-hypophyseal axis for LH is permanently down regulated as with melatonin treatment, but this effect is more pronounced in MET+MT animals. Despite the increase in testosterone titres at 15 and 35 days, the level of the hormone in the postpubertal period was significantly low and, steady right from 45 days. This can suggest reduced sensitivity of Leydig cells or a change in the type of Leydig cells between the pre and post pubertal periods. Clearly, the prepubertal Leydig cells are more responsive as seen from the higher circulating testosterone titres.

Finally, from the observations recorded in the present study, it can be concluded that neonatal melatonin excess coupled with corticosterone deficit has the ability to nullify each other, deleterious effect on tubular functions,

but not on the Leydig cell function. Moreover, it also suggests intricate modulation of the thyroid, adrenal and gonad neuroendocrine axes under combined neonatal hypermelatonemia and hypocorticalism. Further searching investigations on these lines may be more rewarding in understanding the underlying modulatory intricacies and molecular mechanisms.

PLATE – I

Figures 1 – 8: Photomicrographs of sections of testis of control rats.

Figures 1 and 2 : Sections of testis of 35 day old control rats showing interstitium.

Figures 3 and 4 : Section of testis of 45 day of showing advanced stages of spermatogenesis and appearance of sperms in few tubules.

Figures 5 and 6 : Section of testis of 60 day old rats showing well-established spermatogenesis and sperms in lumen.

Figures 7 and 8 : Section of testis of 90 day old rats showing prominent interstitium and fully established spermatogenesis.

Figures: 1, 3, 5, & 7 – 250 x
Figures: 2, 4, 6, & 8 – 400 x

Abbreviations:

I-Interstitium, **L**-Lumen, **st**-spermatids, **S**-sperms,
D-Degeneration,**rs**-round spermatids.

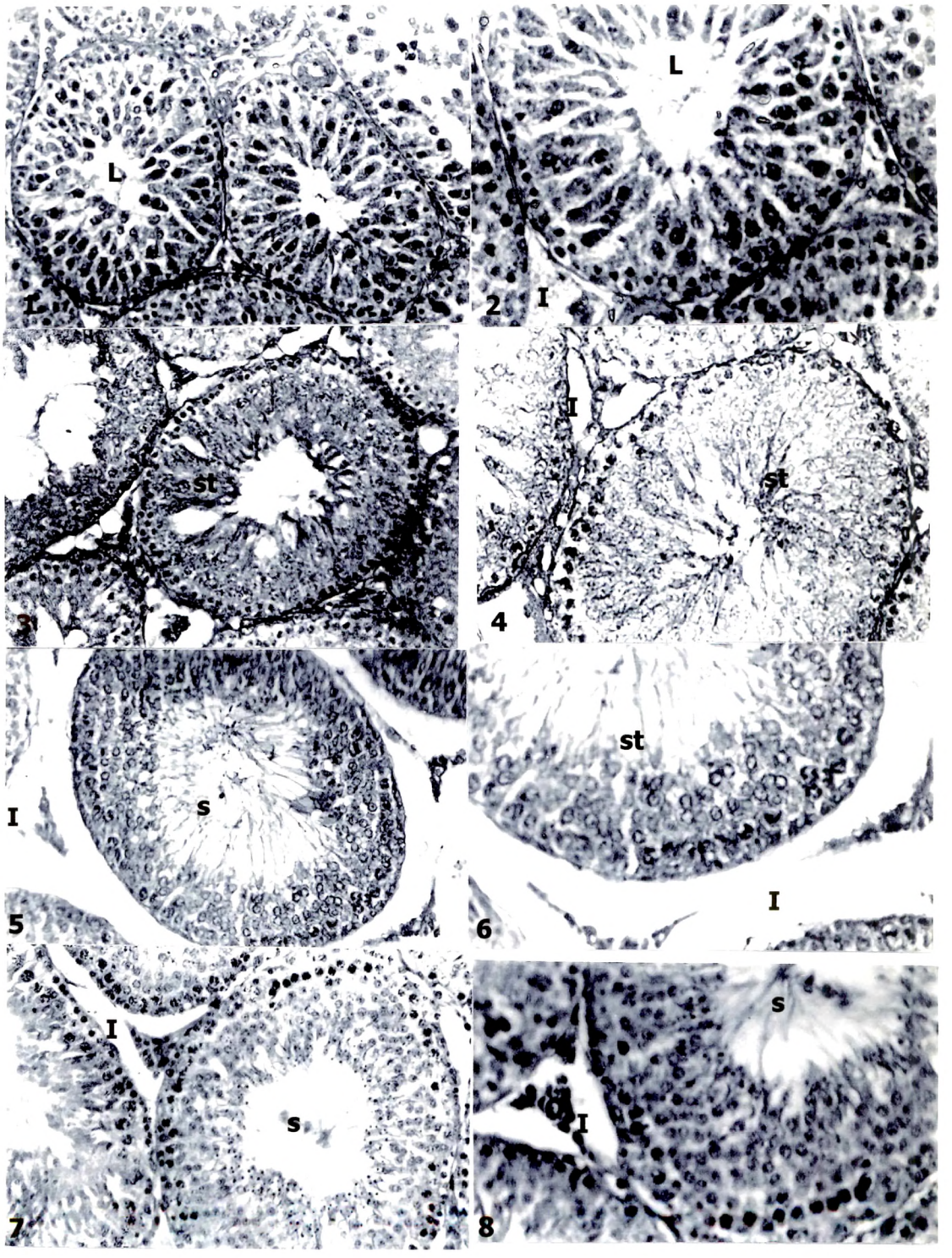

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PLATE – X a

Figures 1 – 8: Photomicrographs of sections of testis in rats treated with Metyrapone and melatonin.

Figures 1 to 4 : Sections of testis of 35 day old MET+MT treated rats showing, degeneration.

Figures 5 to 8 : Sections of 45 day old testis of MET+MT treated rats showing, elongated spermatids, well organized germ cell and presence of sperms.

MET+MT – Metyrapone + melatonin treated rats

Figures: 1, 3, 5 and 7 – 250 x
Figures: 2, 4, 6 and 8 – 400 x

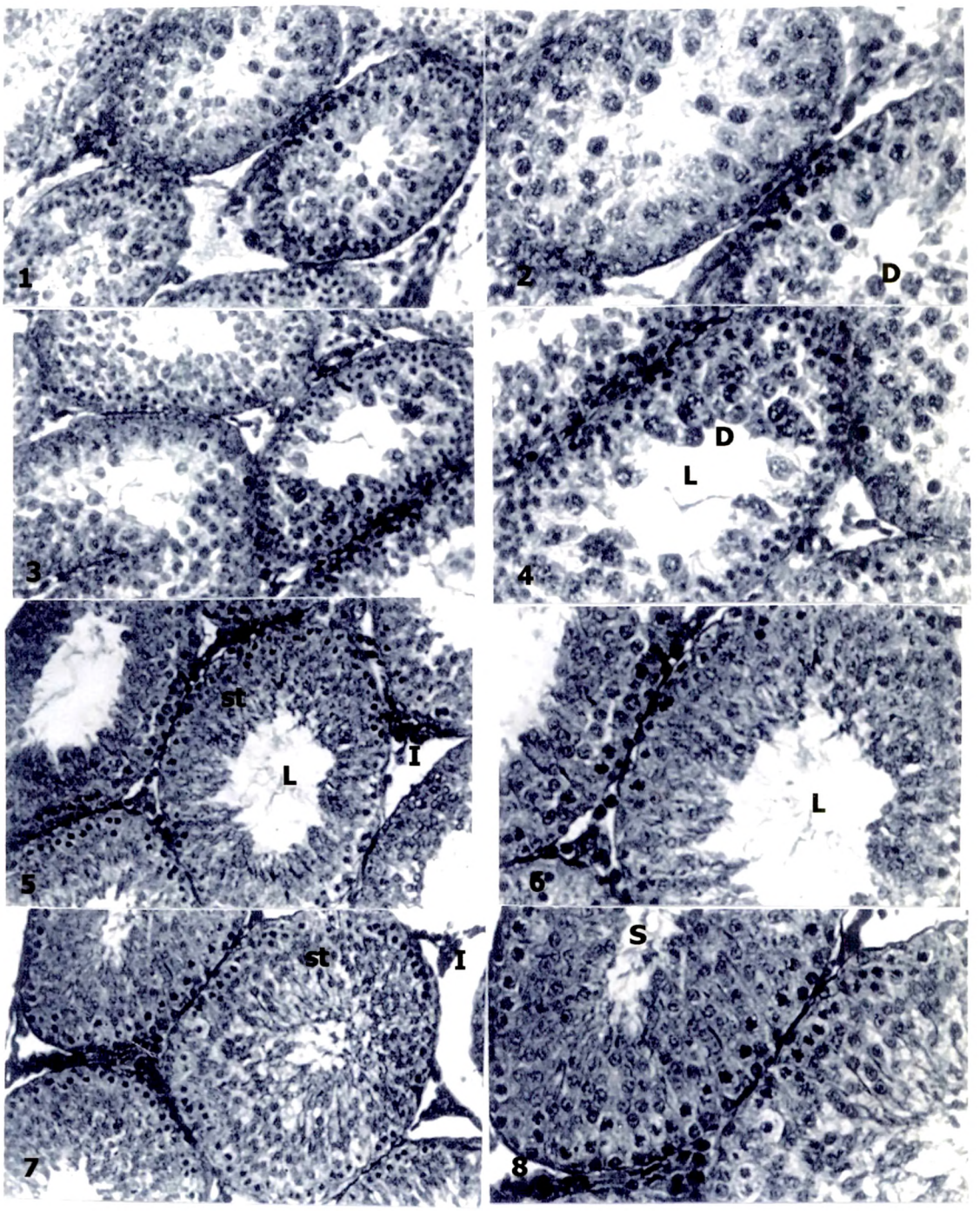
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PLATE – X b

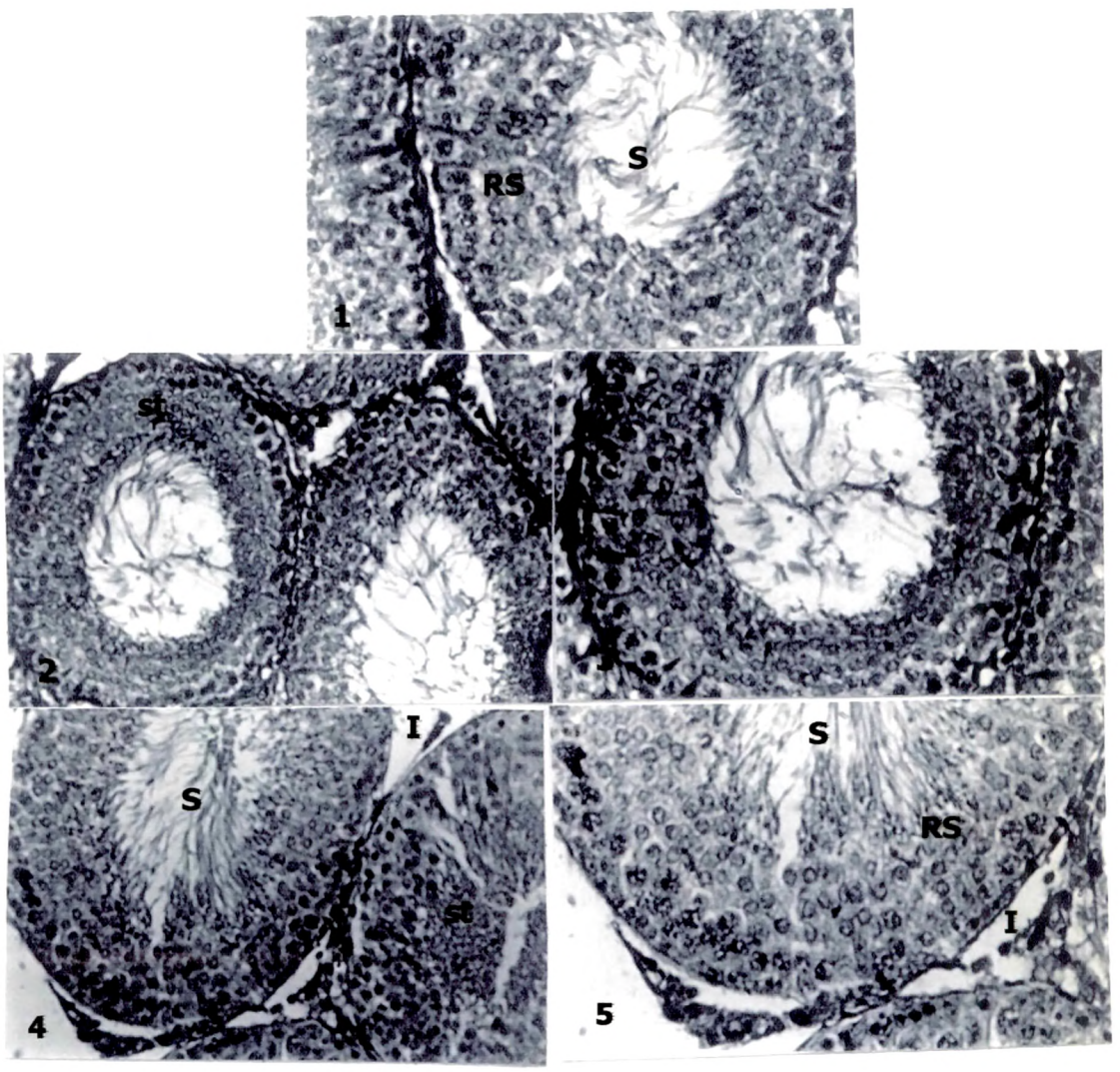
Figures 1 – 5: Photomicrographs of sections of testis in rats treated with Metyrapone and melatonin.

Figures 1 to 3 : Sections of testis of 60 day old MET+MT treated rats showing, fully established spermatogenesis and less. degenerations as compared to earlier stages.

Figures 4 and 5 : Sections of 90 day old testis of MET+MT treated rats showing, well established spermatogenesis and normal sperm density.

MET+MT – Metyrapone + melatonin treated rats

Figures: 2 and 4 – 250 x
Figures: 1, 3 and 5 – 400 x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SUMMARY:

The long-term influence of neonatal hypocorticalism and hypermelatonemia on adult testis structure and functions and serum hormone profiles has been evaluated in the Charles Foster strain of rats. The experimental animals were rendered hypocorticalic by daily injections of metyrapone (2-Methyl, 1,2 di-3-Pyridyl-1-propanone; MET) at the dosage of 0.5 mg/day from day 0 to day 10 and 1 mg/day from day 11 to day 21 and injected with melatonin (MT) in the evening, at the dosage of 40 μg/day from day 0 to day 21 to make them hypermelatonemic. This study is essentially a corollary to the previous study on the effect of neonatal hypercorticalism and hypermelatonemia. The MET+MT animals did not show any significant difference in either the body or testes weights. The testis volume is decreased along with tubular volume, tubular length, basement membrane area and Sertoli cell number. Though the diameter of the seminiferous tubules and the germinal epithelial thickness were increased, actual germ cell count per testis was significantly less but the same was significantly increased per meter length of the tubule. The percentage of germ cell loss was comparable to MT treated rats. The serum T_4 and T_3 levels were reduced but the TSH level was elevated. The levels of LH, T and corticosterone were all significantly decreased. The observed effects on testis are very much similar

to MT treated animals (previous study) and even more pronounced. It is inferred that the effect of melatonin on the growth kinetics of testis constituents is more potentiated in a background of corticosterone deficiency. The influence on the actual number of germ cells per meter length of the tubule, which is quite reverse of that seen in MET treated animals is indicative of the fact that MT has a nullifying influence on MET induced changes. The variations in the hormonal levels recorded in the present case suggest synergistic as well as antagonistic interactions between hypermelatonemia and hypocorticalism on the various hormonal axes.