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Thiola-Induced Effects on Reproductive Organ Morphology and Fertility of Male Rats*

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Effect of Thiola at a dose of 20 mg/kg body weight/day for 30 and 60 days was investigated on reproductive organ weights, their morphology, semen profile and fertility of male rats. The data revealed a decline in the weights of testis, epididymis, vas deferens, accessory sex glands and in cauda epididymidal sperm count and motility in treated rats as compared to control. In general, the decrease was significant by 60 days of treatment. The histoarchitecture of reproductive organs and cauda epididymidal sperm morphology also affected markedly with the duration of treatment. The histometric data showed considerable reduction in seminiferous epithelium/epithelial cell height of testis, epididymis and seminal vesicle of treated rats. These effects finally contributed to loss of fertility in these animals.

Key Words : Male reproductive tract, Morphology, Fertility, Thiola

Introduction

One of the radiochemical protectors, 2-Mercaptopropionylglycine (MPG) is commercially known as Thiola. It gives effective protection on irradiated tissue at very low dose (Uma Devi & Mathur 1981). However, long-term *in vivo* administration of this chemical to adult rats manifested marked alterations in functional status and metabolism of reproductive organs and epididymal spermatozoa (Rao et al. 1985a,b, 1986, Rao 1986a,b). Decreased levels of testosterone in blood and an increased testicular cholesterol content in treated animals resulted in inhibition of androgen synthesis (Rao et al. 1985b, 1986). Immobilization of rat and human spermatozoa *in vitro* was also shown by Rao (1986b) with graded doses of Thiola. But its effect on reproductive organ morphology, semen profile and fertility of rats is not yet studied.

Materials and Methods

Healthy adult male rats (*Rattus norvegicus*) of Charles Foster strain weighing 225–250 g were used for the experiments. They were maintained on a standard diet and water *ad libitum*. The animals were divided into three groups. The control animals received physiological saline as a vehicle. The animals in the other two groups were injected with Thiola i.m. (MPG, Santen Pharmaceuticals Co. Ltd., Japan) at a dose of 20 mg/kg body wt/day for 30 and 60 days respectively. Each group contained 8 to 10 animals.

At the end of the investigations, the animals were autopsied by cervical dislocation and the body and organ weights were recorded. The cauda epididymidal sperm count and motility were made using the method of Prasad et al. (1972) and expressed as million sperms/ml and percentage (%) motility respectively. The Scanning Electron Microscopic (SEM) study on sperm morphology was done according to the techinque of Rao and Chinoy (1983) and fertility test by adopting the method of Chinoy et al. (1982a).

For histological studies, small pieces of cauda and caput epididymides, vas deferens, testis and seminal vesicles were fixed in Bouin's fluid, dehydrated and embedded in paraffin wax, sectioned at 4–5 μm and

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stained with haematoxylin and eosin. The histometric data for epithelial cell height, diameter of Leydig cells and their nuclei as well as tubular structures were carried out using the ocular eye-piece and micrometer scale and expressed as μm (Rao & Chinoy 1984). Twenty to thirty readings were taken for each parameter and tissue. The data were statistically analyzed using student's 't'-test.

Results

Body and organ weights: The weights of various reproductive organs were found to reduce in rats treated with Thiola for 30 and 60 days as compared to control animals. The fall in organ weights was significant in rats treated only for 60 days (testis and vas deferens, $P<0.05$; caput, $P<0.001$, cauda, $P<0.01$; seminal vesicle and coagulating gland, $P<0.001$). Body weights were not changed by this treatment (table 1).

Sperm count, motility and fertrility rate: The number of sperms in testis and cauda was found to decrease with the duration of treatment. The reduction in cauda epididymidal sperm count and motility was significant ($P<0.01$); $P<0.001$) by 60 days of Thiola administration. The fertility rate was 95% negative (−ve) in two months treated rats in comparison to other groups (table 2).

SEM study on sperm morphology: The normal rat cauda epididymidal sperms had characteristically sickle-shaped heads and the tail-end-piece was straight (plate 1, figures 1 & 2). In Thiola received rats, sperms from cauda exhibited stripping off of the cell membrane and damage in neck region (plate 1, figure 3) and decapitation (plate 1, figure 4).

Histology: The morphology of caput and cauda epididymides of rats treated for 30 and 60 days with Thiola showed marked changes in tubular shape as compared to controls. Degeneration of pseudostratified epithelium, pyknotic nuclei and an increased intertubular space filled with attenuated connective tissue fibers were found in treated rat cauda (plate 2, figures 1 & 2). Vacuolization in epithelial cell layer and cellular debris in tubular lumen were also noted in caput epididymidis of Thiola-injected rats (plate 2, figures 3 & 4). In distal vas deferens, the epithelial cell layer was distorted and slaughed off into the lumen (plate 2, figures 5 & 6).

Histometry: The histometric data showed significant reduction in germinal epithelium/epithelial cell height of testis, cauda, caput and seminal vesicle (testis, $P<0.001$; cauda, $P<0.05$; caput, $P<0.02$; SV, $P<0.001$) in treated rats as compared to controls. The tubular diameter of testis and epididymis also considerably ($P<0.001$) reduced by 60 days of treatment. The decrease in Leydig cell and its nuclear diameters was also found to be significant ($P<0.02$). However, the muscle layer of seminal vesicle (SV) did not show any reduction in thickness (table 3).

Table 1 *Body and organ weights of control and Thiola-treated male rats*

Weight (mg)	Control	30 days	60 days
Body weight (g)	228 ± 34	222 ± 17	218 ± 11
Testis	1370 ± 0.03	1220 ± 0.21	1100 ± 0.12
Cauda	170 ± 23	154 ± 7.97	128 ± 11.3
Caput	253 ± 5.8	183 ± 4.95	156 ± 7.66
Distal vas	42 ± 8.2	38 ± 0.92	29 ± 2.54
Proximal vas	19 ± 1.1	12 ± 1.00	10 ± 0.70
Seminal vesicle	300 ± 45	229 ± 12.7	203 ± 34
Ventral prostate	185 ± 15	168 ± 18	163 ± 8.1
Coagulating gland	54 ± 6.4	48 ± 4.4	22 ± 2.8

Values are Mean ± SE

Table 2 *Sperm motility and count of cauda and fertility rate in control and Thiola-treated rats*

Parameter	Control	30 days	60 days
Testicular sperm count (mill/ml)	4.5 ± 0.05	2.91 ± 0.72	2.60 ± 0.11
Cauda sperm count (mill/ml)	50 ± 6.11	41 ± 1.61	35 ± 5.50
Motility (%)	72 ± 3.52	45 ± 2.71	*14 ± 0.70
Fertility rate	95% (+ve)	50% (−ve)	95% (−ve)

Values are mean ± SE; *Sluggishly motile sperms observed; +ve = Positive; −ve = Negative

Table 3 *Histometric data of reproductive organs in control and treated rats*

Tissue	Parameter (μm)	Control	60 days
Testis	T.D.	319 ± 10	*212 ± 28
	E.H.	81 ± 2	Complete depopulated cells
	L.D.	7 ± 0.3	4.36 ± 0.2
	L.N.D.	3.1 ± 0.02	1.24 ± 0.1
Cauda	T.D.	386 ± 10	283 ± 21
	E.H.	20 ± 0.8	11 ± 0.6
Caput	T.D.	388 ± 14	301 ± 18
	E.H.	25 ± 0.9	14 ± 1.3
Seminal vesicle	M.L.T.	89 ± 3.6	78 ± 6.4
(SV)	E.H.	15 ± 0.7	*8 ± 0.3

Values are mean ± SE; *T.D.*, Tubular diameter; *E.H.*, Germinal epithelium/epithelial cell height; *L.D.*, Leydig cell diameter; *L.N.D.*, Leydig cell nuclear diameter; *M.L.T.*, Muscle layer thickness; *Damaged seminiferous epithelium/SV epithelium

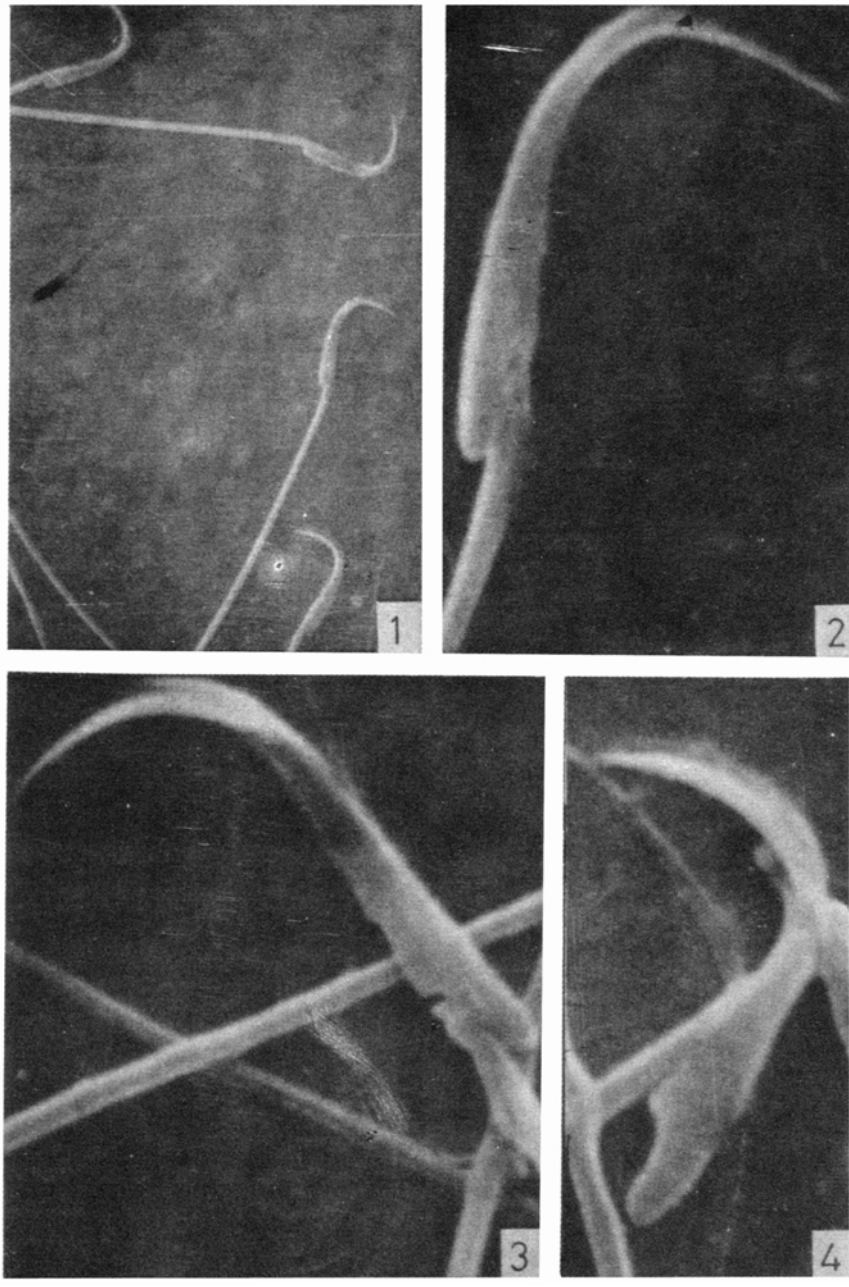
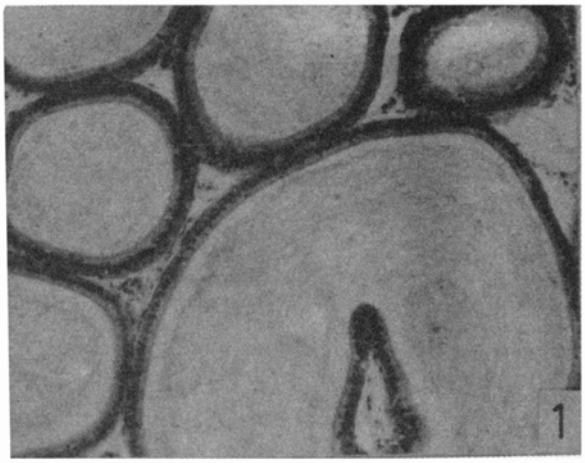
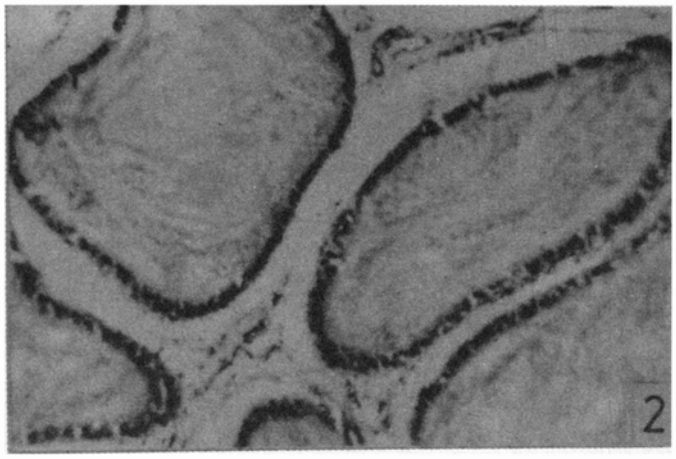
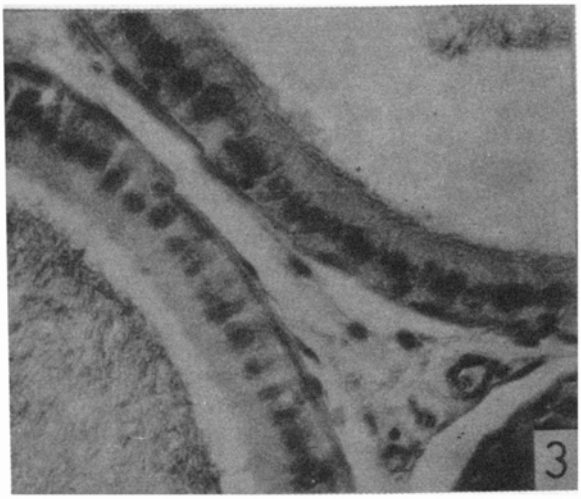
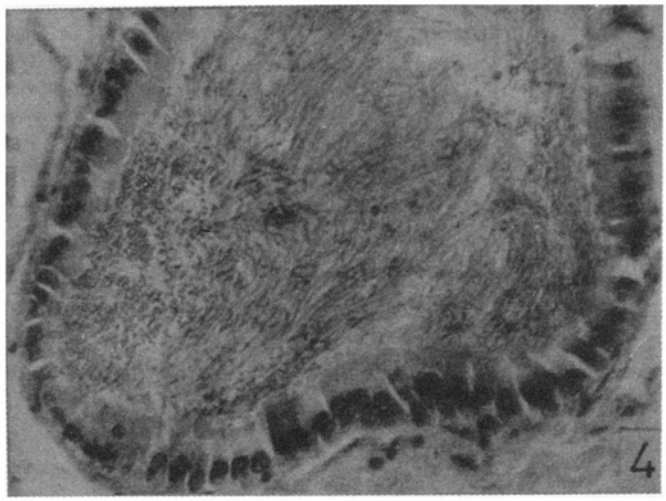


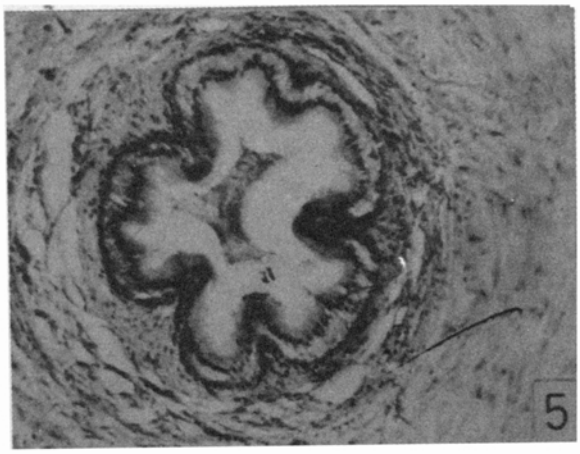
Plate 1 figures 1–4. 1, SEM of normal rat cauda epididymidal spermatozoa (×450); **2,** SEM of magnified view of sperm head (×1300); **3,** SEM of 60 days treated rat sperm from cauda (×1250); **4,** SEM of cauda epididymidal sperm showing decapitation (×1550)











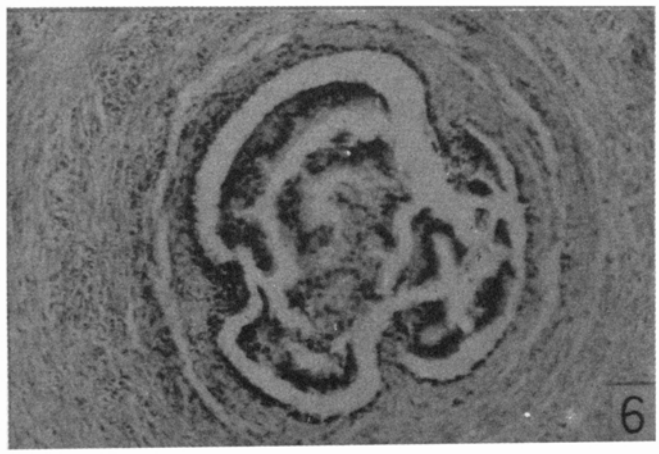


Plate 2 figures 1–6. 1, Transverse section (TS) of normal rat cauda showing pseudostratified epithelium with sperm bundles in lumen (×98); **2,** TS of rat cauda treated for 60 days with Thiola (×96); **3,** TS of caput epididymidis of control rat (×256); **4,** TS of caput of rat treated with Thiola for 30 days indicating cellular debris in lumen (×256); **5,** TS of distal vas deferens of control rat showing star shaped lumen with steriocilia (×112); **6,** TS of distal vas deferens of 60 days treated rat (×110).

Discussion

Reports from our laboratory indicate diminished circulating levels of testosterone in Thiola-administered rats, leading to androgen-deprived effect to target organs. It is supported by a significant increase in testicular cholesterol content. The androgen-sensitive biochemical parameters in testis and vas deferens also decreased (Rao et al. 1986). These effects in turn caused loss of organ weights in treated rats, as observed in this study. The decrease in Leydig cell diameter noted in this report, is probably due to reduced testicular androgenicity. Similar effects are also reported with other radioprotective chemicals in laboratory animals (Shah 1984). Apart from the regressive changes in epididymis and vas deferens, androgen deficiency also caused marked reduction of the tubular diameter and in number of spermatozoa in cauda epididymidis. The changes in composition of epididymal plasma in treated animals have adversely affected sperm survival and maturation. It is well known that the normal secretory activity of the epididymal epithelium and testicular spermatogenesis are entirely controlled by circulating androgens (Mann & Lutwak-Mann 1981). The regressive changes were also evident in seminal vesicle of treated rats which further support the antiandrogenic effect of Thiola.

The present findings also suggest the decrease in epididymal and testicular sperm count to certain extent due to the probable antispermatogenic effect of Thiola. The significant reduction in sperm motility and an increased abnormal sperm morphology in treated rat cauda in this study are related to alterations in their structural and biochemical components. The decreased succinate dehydrogenase and ATPase activities also suggested changes in sperm oxidative/energy metabolism by this treatmenrt (Rao 1986a). These factors have probably contributed to loss of fertility in Thiola-treated animals. This is consistent with the findings of Rao et al. (1985a,b, 1986). It is thus obvious that the treatment with Thiola induces antiandrogenic and antifertility effects in rats.

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