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The Effects of Tris(2,3-Dibromopropyl)Phosphate on the Reproductive System of Male Rats

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ABSTRACT

Tris(2,3-dibromopropyl)phosphate (TRIS) administered intraperitoneally to adult, male Sprague-Dawley rats for 12 weeks at concentrations of 85.1 to 340.5 mg/week caused significant, dose-related declines in the weights of the testes, epididymides, and seminal vesicles. Sperm production by the testes and sperm storage in the epididymides were also reduced. Although prostate weight declined, the effect was not correlated with the dose of TRIS. Neither serum concentrations of testosterone nor the testicular capacity to produce testosterone in vitro were affected by any dose of TRIS.

INTRODUCTION

TRIS (2,3-DIBROMOPROPYL)PHOSPHATE, TRIS, was used as a flame retardant in a variety of fabrics, including acetates and polyesters made into children's sleepwear prior to 1977. The use of TRIS was discontinued after studies indicated numerous toxic effects, including mutagenicity and carcinogenicity, which could potentially jeopardize human health.[1] In one of these studies, dermal exposure to TRIS caused rabbit testes to shrink and the seminiferous tubules to take on an abnormal appearance.[2] However, neither the extent of toxic effects of TRIS on the organs of the male reproductive system nor the potency of TRIS in causing reproductive effects were determined.

The current investigation was undertaken to ascertain the amounts of TRIS necessary to produce significant effects on spermatogenesis, to examine other organs of the male reproductive tract for effects of TRIS, and to examine possible mechanisms for TRIS's reproductive toxicity.

MATERIALS AND METHODS

Tris (2,3-dibromopropyl)phosphate of high purity (sample PZ-668) was obtained from Chem Service (West Chester, PA).

Adult male Sprague-Dawley rats, 56–60 days of age (250 g, Dominion) were purchased and housed in the Johns Hopkins University School of Hygiene and Public Health under a 14:10 light-dark cycle, at 24°C. The rats were provided Purina Rat Chow and water ad libitum.

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Experimental design

The rats (66) were divided into three experimental groups. Six rats served as untreated controls. Six rats were injected with 0.1 ml of propylene glycol intraperitoneally on Mondays, Wednesdays, and Fridays as injected controls. Fifty-four rats, 6 rats for each of 9 different doses, were injected intraperitoneally Mondays, Wednesdays, and Fridays with 0.1 ml of TRIS:propylene glycol mixtures (113.5, 56.8, 28.4, 14.2, 7.1, 3.5, 1.8, 0.9, 0.4 mg TRIS/injection) for a period of 72 days. At the four highest concentrations, the TRIS was not completely miscible with the propylene glycol, and the mixture had to be shaken for 30 seconds to obtain an emulsion just prior to injection. TRIS completely dissolved in the propylene glycol at the other five concentrations.

All rats were treated for a minimum of 72 days (6 cycles of the germinal epithelium) before being killed. At the end of treatment, each rat was weighed and killed by decapitation, and the blood was collected for analysis of serum testosterone concentrations. Serum testosterone concentrations were determined by radioimmunoassay (RIA).[3] The testes were removed from the rats and weighed. One testis was perfused in vitro with an artificial medium containing 100 ng/ml ovine LH (S 21, National Pituitary Agency) to maximally stimulate testosterone output.[4] Testosterone in the perfusate was assayed using high-performance liquid chromatography.[5] Following perfusion, the testis was decapsulated, and homogenized in a Waring blender.[6] The homogenate was placed in a hemacytometer, and condensed spermatids and spermatozoa were counted at $\times 400$ under a phase contrast microscope.

The contralateral testis was weighed, perfusion fixed, and fragmented for later histological examination.[4] The weights of the ventral prostate and the seminal vesicles, following expression of the seminal fluid, were also determined.[7] A droplet of sperm from the tail portion of one epididymis was diluted in 5 ml of Tyrode's buffer[7] at 37°C and examined for the percentage of motile sperm. The motility index was determined according to Sherins and Howards.[8] One drop of undiluted sperm from the epididymal tail was placed on a microscope slide, smeared, and allowed to air dry. The sperm morphology[8] was examined at $\times 400$ under a phase contrast microscope. The contralateral epididymis was divided into two sections, the head-body and the tail, which were weighed separately. The tail was homogenized for counting sperm.[9]

Histology

Fixed testicular fragments were washed in cacodylate buffer, dehydrated, and embedded in eponaraldite.[10] Five fragments from each testis were embedded and thick sectioned at 1 micron. The sections were stained with toluidine blue and observed using a light microscope.

Statistical analysis

The results were analyzed using a one-way analysis of variance, Duncan's multiple range test,[11] and least squares regression analysis of the data. The data were fitted to quadratic equations to approximate a threshold effect of the toxin.

RESULTS

Analysis of variance and Duncan's multiple range test of the results in Table 1 indicated that treatment with TRIS significantly ($P < 0.05$) reduced animal weights compared to controls. The effect on animal weight was correlated with the dose of TRIS. None of the treated animals displayed any clinical symptoms, although 2 rats died during the second week of treatment with 340.5 mg/week.

Male reproductive tract

TRIS caused a significant ($P < 0.05$) decrease in testis weight, prostate weight, weight of expressed seminal vesicles, and epididymal weights (Table 2). The declines in reproductive organ weights, except for prostate weight, correlated with the dose of TRIS.

TABLE 1. EFFECT OF TRIS ON BODY WEIGHT OF RATS

Treatment	*Dose (mg/week)*	*Final body weight*[a] *(g)*
Control		472 ± 18
Injected control		451 ± 25
TRIS	1.3	477 ± 14
	2.7	485 ± 17
	5.4	439 ± 22
	10.7	458 ± 11
	21.3	445 ± 11
	42.6	421 ± 4
	85.1	412 ± 8[b]
	170.2	384 ± 14[b]
	340.5	339 ± 31[b,c]
r		0.74

[a]Mean ± SEM of 6 replicates.
[b]Significantly different ($P < .05$) from both control values as determined by Duncan's multiple range test.
[c]$n = 4$.
r, correlation coefficient for least squares regression analysis of the data. The data were fitted to a quadratic equation to approximate a threshold effect of the toxin.

TABLE 2. EFFECT OF TRIS ON WEIGHTS OF MALE REPRODUCTIVE TRACT ORGANS

Treatment	*Dose (mg/week)*	*Testes weight*[a] *(g)*	*Prostate weight (g)*	*Vesicle weight (g)*	*Epididymal weight (g)*
Control		3.48 ± 0.12	0.74 ± 0.06	0.64 ± 0.03	0.58 ± 0.02
Injected control		3.67 ± 0.13	0.62 ± 0.06	0.67 ± 0.03	0.62 ± 0.01
TRIS	1.3	3.56 ± 0.10	0.58 ± 0.03	0.62 ± 0.03	0.60 ± 0.02
	2.7	3.89 ● 0.11	0.64 ± 0.06	0.58 ± 0.03	0.63 ± 0.03
	5.4	3.56 ± 0.16	0.67 ± 0.09	0.60 ± 0.03	0.56 ± 0.01
	10.7	3.62 ± 0.14	0.62 ± 0.04	0.58 ± 0.04	0.57 ± 0.01
	21.3	3.44 ± 0.09	0.64 ± 0.05	0.59 ± 0.04	0.58 ± 0.02
	42.6	3.43 ± 0.20	0.53 ± 0.06[b]	0.51 ± 0.05[c]	0.52 ± 0.03
	85.1	3.04 ± 0.15	0.48 ± 0.05[b]	0.52 ± 0.04[c]	0.50 ± 0.03[c]
	170.2	2.13 ± 0.17[c]	0.50 ± 0.05[b]	0.52 ± 0.04[c]	0.50 ± 0.03[c]
	340.5[d]	1.53 ± 0.24[c]	0.47 ± 0.02[b]	0.38 ± 0.02[c]	0.28 ± 0.02[c]
r		0.86	0.46	0.66	0.87

[a]Mean ± SEM of 6 replicates.
[b]Significantly different ($P < 0.05$) from untreated control as determined by Duncan's multiple range test.
[c]Significantly different ($P < 0.05$) from both control values as determined by Duncan's multiple range test.
[d]$n = 4$.
r, correlation coefficient for least squares regression analysis of the data. The data were fitted to a quadratic equation to approximate a threshold effect of the toxin.

Histological examination of the testes revealed affected seminiferous tubules contiguous with tubules of normal appearance (Fig. 1). The affected tubules contained very few germinal cells, and the macrophages in the interstitium of the affected testes appeared to be phagocytically active (Fig. 2). At the light level, Leydig cells in the interstitium appeared normal. In some histological sections, cells similar in appearance to macrophages were seen inside the affected seminiferous tubules (Fig. 3).

Spermatogenesis and sperm motility

TRIS significantly ($P < 0.05$) reduced sperm production by the testes and diminished the sperm content of the epididymal tail. These effects of TRIS correlated with the dose (Table 3). TRIS also had a significant ($P < 0.05$) effect on the percentage of motile sperm and the motility index (Table 4). However, these effects were not correlated with dose. Examination of sperm smears at $\times 1000$ revealed no obvious morphological abnormalities of the sperm heads.

Endocrinology

TRIS had no significant effect on the serum concentration of testosterone or on the in vitro testicular capacity for testosterone secretion (Table 5).

DISCUSSION

Previous studies have shown that TRIS has an acute LD_{50} in rats of 1.6 g/kg.[1] Chronic dosing produces effects on the kidneys and liver, in addition to the reproductive system.[1] The half-life of

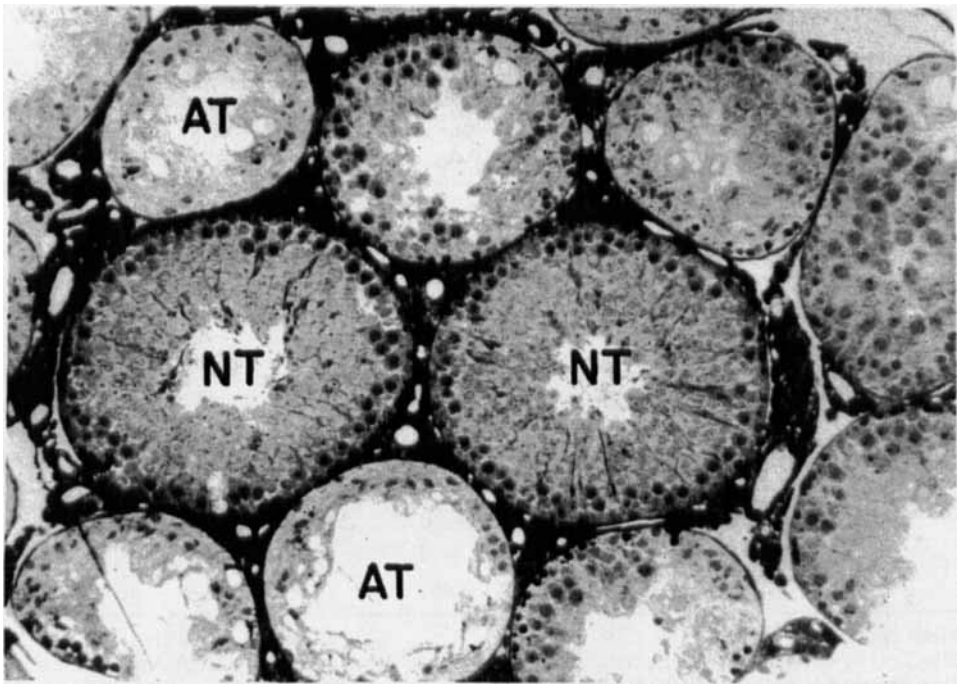

FIG. 1. Cross-section of seminiferous tubules from the testis of a rat injected intraperitoneally with 340.5 mg TRIS/week. Affected tubules (AT), contiguous with normal tubules (NT), exhibit a marked loss of the germinal epithelium. $\times 100$.

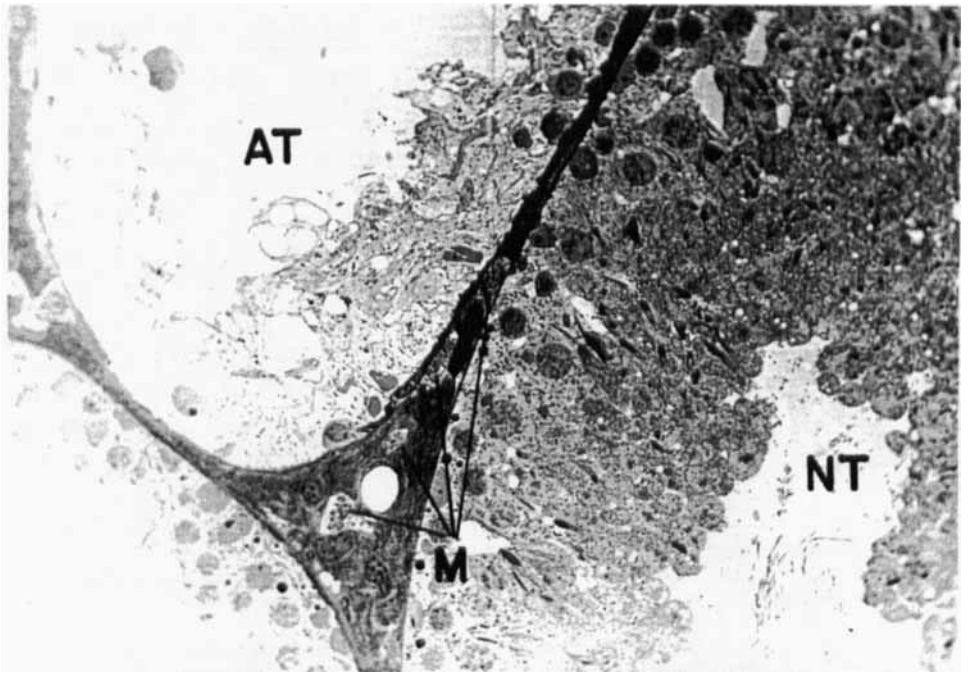

FIG. 2. Cross-section of seminiferous tubules from the testis of a rat injected intraperitoneally with 340.5 mg TRIS/week showing enhanced phagocytic activity of the macrophages (M) in the interstitium adjacent to affected tubules (AT). Only the Sertoli cells appear to remain inside the affected tubules. ×400.

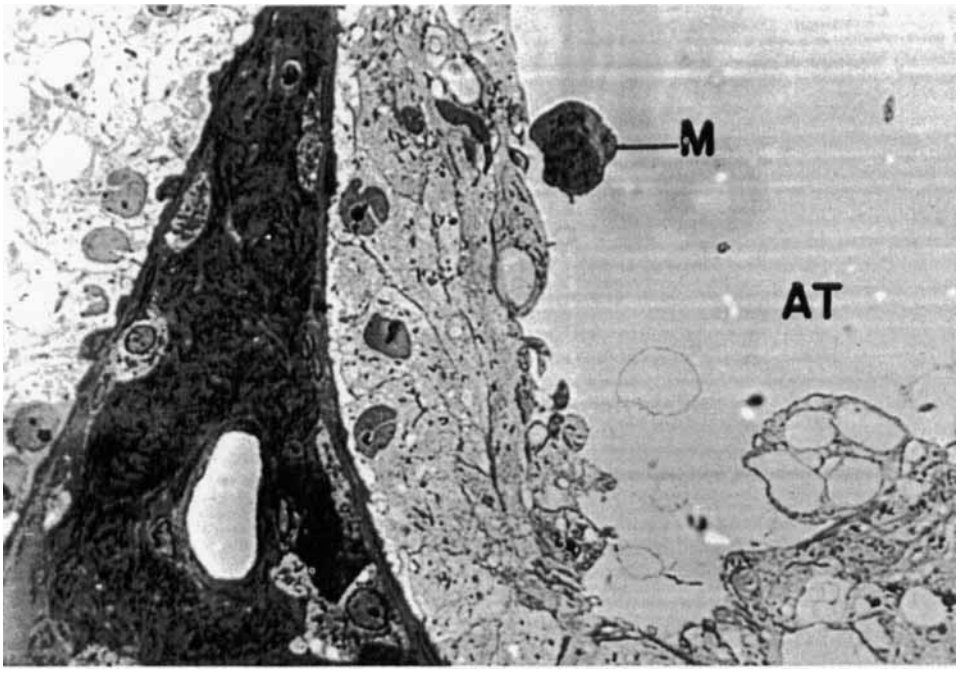

FIG. 3. Cross-section of affected seminiferous tubule from the testis of a rat injected with 340.5 mg TRIS/week showing what appears to be a macrophage inside the seminiferous tubule. ×800.

TABLE 3. EFFECTS OF TRIS ON SPERM CONTENT OF TESTES AND EPIDIDYMIDES

Treatment	*Dose (mg/week)*	*Sperm/testis*[a] *(× 10⁻⁶)*	*Sperm/epididymis (× 10⁻⁶)*
Control		203 ± 17	193 ± 14
Injected control		195 ± 17	197 ± 18
TRIS	1.3	217 ± 13	184 ± 20
	2.7	237 ± 22	186 ± 20
	5.4	217 ± 16	182 ± 13
	10.7	215 ± 19	163 ± 13
	21.3	212 ± 14	164 ± 18
	42.6	189 ± 9	141 ± 18
	85.1	178 ± 15	117 ± 26[b]
	170.2	117 ± 32[b]	78 ± 21[b]
	340.5	36 ± 18[b,c]	7 ± 3[b,c]
r		0.69	0.81

[a]Each value represents the mean ± SEM for 6 replicates.
[b]Significantly different ($P < 0.05$) from both control values as determined by Duncan's multiple range test.
[c]$n = 4$.
r, correlation coefficient for least squares regression analysis of the data. The data were fitted to a quadratic equation to approximate a threshold effect of the toxin.

TABLE 4. EFFECTS OF TRIS ON SPERM MOTILITY

Treatment	*Dose (mg/week)*	*% Motile*[a]	*Motility index*
Control		60 ± 3	2.0 ± 0
Injected control		55 ± 5	2.0 ± .3
TRIS	1.3	63 ± 4	2.0 ± 0
	2.7	61 ± 5	1.7 ± .2
	5.4	60 ± 3	1.7 ± .2
	10.7	58 ± 5	1.8 ± .2
	21.3	63 ± 3	1.8 ± .2
	42.6	67 ± 2	2.2 ± .2
	85.1	63 ± 2	1.8 ± .2[b]
	170.2	49 ± 4	1.5 ± .2[b]
	340.5	35 ± 15[b,c]	1.3 ± .5[b,c]
r		0.47	0.36

[a]Each value represents the mean ± SEM for 6 replicates.
[b]Significantly different ($P < 0.05$) from both control values as determined by Duncan's multiple range test.
[c]$n = 4$.
r, correlation coefficient for least squares regression analysis of the data. The data were fitted to a quadratic equation to approximate a threshold effect of the toxin.

TRIS in rats has been found to be 6 hours.[11] Although another study demonstrated that 50% of TRIS-derived radioisotope was not eliminated from rats for 24 hours.[12]

The diminished size of rat testes produced in response to TRIS injections in this study is consistent with the published effects of dermal exposure of rabbits to high concentrations of the toxin.[2] The shrinkage of the testes seems to result from diminished sperm production (Table 3). Histological ex-

TABLE 5. EFFECTS OF TRIS ON SERUM TESTOSTERONE CONCENTRATIONS AND THE TESTICULAR PRODUCTION OF TESTOSTERONE

Treatment	*Dose (mg/week)*	*Serum testosterone (ng/ml)*[a]	*Testosterone secretion (μg/testis/hour)*
Control		2.25 ± 0.34	5.21 ± 0.95[b]
Injected control		1.73 ± 0.27	4.47 ± 0.50[b]
TRIS	1.3	1.60 ± 0.49	5.92 ± 0.44[b]
	2.7	1.53 ± 0.25	6.09 ± 0.69[b]
	5.4	2.89 ± 0.39	5.70 ± 0.61[c]
	10.7	2.44 ± 0.47	5.87 ± 0.67[b]
	21.3	2.04 ± 0.64	6.64 ± 1.40[b]
	42.6	1.83 ± 0.21	5.98 ± 0.85[b]
	85.1	2.35 ± 0.58	5.61 ± 1.13[c]
	170.2	1.77 ± 0.35	6.10 ± 0.75[b]
	340.5	1.48 ± 0.32[c]	5.24 ± 1.55[c]
r		0.20	0.08

[a]Mean ± SEM for 6 replicates.
[b]$n = 5$.
[c]$n = 4$.
r, correlation coefficient for least squares regression analysis of the data. The data were fitted to a quadratic equation to approximate a threshold effect of the toxin.

amination of the affected testes showed that some, but not all, of the seminiferous tubules appeared to contain only Sertoli cells (Fig. 1). The affected tubules in the rat were similar in appearance to the affected tubules reported in the rabbit.[2] In the rat, the apparent phagocytic activity of the macrophages in the interstitium contiguous with these Sertoli cell-only tubules coupled with the presence of macrophages inside the tubules suggest that a breakdown of the blood–testis barrier may possibly have taken place. If this were to occur, the loss of the germinal epithelium might be the result of an autoimmune response of the body to the germinal cells in the seminiferous tubules.[13] Alternatively, TRIS may be cytotoxic to the cells of the seminiferous tubules in a manner similar to its effects on the renal tubules.[2]

TRIS also affects the size of the sex accessory organs of the male rat (Table 2) and decreases epididymal sperm content (Table 3). Epididymal sperm content appears to be affected at even lower concentrations of TRIS than testicular sperm content. Although sperm motility is adversely affected (Table 4), comparison of the morphology of sperm in treated vs untreated animals revealed no obvious differences at the light level.

The toxic effects of TRIS on spermatogenesis, sperm motility, and male reproductive organ weights suggest a mode of action involving the disruption of the pituitary-gonadal hormonal axis. However, if pituitary gonadotropins were affected, the testicular capacity for testosterone secretion should decline[10] along with serum testosterone concentrations.[7,9] Despite a 50% reduction in the size of the testes, the in vitro testicular capacity for testosterone secretion is unaffected by TRIS (Table 5). Serum testosterone concentrations do not show any significant effect either. Further, if disruption of hormone levels were the mechanism of TRIS toxicity, all of the seminiferous tubules would have been affected, and both spermatogonia and spermatocytes would have been present.[14] TRIS, however, has a differential effect on the tubules, and the affected tubules contain only Sertoli cells and a few spermatogonia. Thus, TRIS does not appear to affect reproductive endocrinology.

TRIS does seem to effect a breakdown of the blood–testis barrier in some of the seminiferous tubules. The mechanism by which this can be done is unknown. Whether the effects of TRIS on the other organs of the male reproductive tract of rats will be traced to that same unknown mechanism remains a subject for investigation.

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