Effect of Triethylenemelamine on Reproductive Capacity of Mouse Spermatozoa.* (25514)

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Triethylenemelamine (TEM) produces temporary sterility in male rats(1) as evidenced initially by early reduction in size of litters produced by females with which they are mated, and later by complete sterility. Smaller litters are accounted for by radiomimetic action of TEM in causing germinal epithelium of testes to produce so called(1) "sterile" spermatozoa which induce early embryonic death(1,2). Depending upon dose, TEM can affect germinal epithelium by causing histologically demonstrable damage to spermatogonia or by altering germinal cells in more subtle fashion so that spermatozoa are unable to produce viable offspring(2). Information on duration of spermatogenesis and passage of free spermatozoa(3,4) and available data on intervals between drug treatment and mating(1,2) support the view that the adverse effect on male fertility may be caused by injury to spermatozoa, as well as germinal epithelium. This communication presents evidence of this direct effect of TEM on reproductive capacity of mouse spermatozoa in reducing not only litter size but also per cent fertility, as tested by artificial insemination.

Materials and methods. Forty-four C57 male brown mice weighing 26 to 29 g were injected intraperitoneally each of 5 consecutive days with 0.006 mg TEM in 0.25 ml normal saline. This corresponds to the dose which reduced fertility and caused structural alterations in rat testes(2). Thirty-four were used in artificial insemination, 10 for measurements of density and additional observations on motility. Artificial insemination, after Kile(5), was used to evaluate reproductive capacity of spermatozoa from control and TEM treated males. Females of known fertility, kept in cyclic estrus by controlled lighting, were inseminated one to 3 hours following mating with vasectomized males. Both sexes were of BALB/c strain mice. After mid-ventral laparatomy, 0.1 ml of the spermatozoal suspension from a C57 male was introduced through the wall of each uterine horn with a 27 gauge hypodermic needle. The genetic label of eye color, carried by the spermatozoa of the C57 brown males, served as detectable insurance that all fetuses, both control and experimental, resulted from artificial insemination. Inseminated females were killed by etherization and autopsied on what was the nineteenth day of gestation in pregnant mice. TEM treated males were sacrificed by etherization 3 days after last injection. Testes were removed, fixed in Bouin's solution, and subsequently processed for histological observations on 5 μ paraffin sections stained by PAS - Weigert's hematoxylin technic. The cauda epididymides and the pair of ductus deferentes were removed from each animal. Each pair of structures was placed separately in depression slides containing 0.2 ml of a yolk-citrate-Locke's (YCL) medium(6). Spermatozoa were obtained from the epididymides by mincing with fine scissors, those from the ductus deferentes by stripping with blunt flat forceps. Following a 5 minute wait, each suspension of free spermatozoa was drawn up into a 0.25 ml syringe for insemination. The remainder of the sample was used in triplicate readings at 37°C on per cent motility. Type of motility and obvious abnormalities were noted. Density measurements were made with a blood counting chamber(7). Forty-nine untreated C57 brown mice served as controls.

From each of 8 mice, spermatozoa from the left cauda epididymis and ductus deferens were placed in YCL and those from the right side placed in YCL which contained the same concentration of TEM as given in a single injection to the other experimental mice. The

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TABLE I. Results of Artificial Insemination with Spermatozoa from Male Mice Treated with Triethylenemelamine.*

Spermatozoa	No. of females Insem-inated	Preg-nant	% fer-tility‡	No. of fetuses Total	Avg litter
Treated					
Ductus deferentes	34	3	9	5	1.7
Cauda epididymides	21	7	33	12	1.7
Total	55	10	18	17	1.7
Untreated†					
Ductus deferentes	30	20	67	100	5.0
Cauda epididymides	28	15	53	72	4.8
Total	58	35	60	172	4.9

* Inj. intraper. with 0.2 mg/kg TEM for each of 5 consecutive days.
† Control group.
‡ Nearest whole number.

type and per cent motility of spermatozoa from both sides of the same animals were then compared.

Results. Artificial insemination with spermatozoa from the cauda epididymides and ductus deferentes of mice injected each of 5 consecutive days with a dose of TEM equivalent to 0.2 mg/kg body weight, resulted in a marked decrease in per cent fertility, as well as reduced litter size. Inseminations with spermatozoa from the ductus deferens resulted in a greater lowering of per cent fertility than those from the epididymis (Table I). Per cent motility and density of spermatozoa from animals so treated with TEM were within the range of values found in control mice (Table II). No evidence of induced structural abnormalities in spermatozoa was observed. TEM treatment caused an average loss of one gram of body weight.

Histological comparisons of testes from untreated and TEM treated males revealed no TEM induced structural deviations from normal spermatogenesis.

The type, speed, and per cent motility of spermatozoa treated *in vitro* with the same concentration of TEM as used in one injection, appeared unchanged from the normal in the comparisons between treated and untreated cells from the same animal.

Discussion. We agree that an adverse effect of TEM on spermatozoa is reflected in reduced litter size of inseminated females(8). In addition, we find that per cent fertility also is diminished although number, motility, and appearance of spermatozoa remain unchanged, in the absence of testicular structural abnormalities.

Eight days elapsed between first injection of TEM and insemination, while 7 to 8 days are required for spermatozoa to traverse the epididymis in passing from testis to ductus deferens(4). Thus, inseminated samples from the ductus deferentes probably contained spermatozoa which were in the ductus deferentes, in the epididymides and in the testes, at the time TEM was first injected. Epididymal samples inseminated probably included spermatozoa which were late spermatids at this time. Therefore, results (Table I) do not support the view(8) that spermatids are more sensitive to TEM than are spermatozoa. This may be explained if the stage at which spermatids are treated is critical. Early and not late spermatids may be more sensitive than spermatozoa to TEM.

A double action of TEM in reducing male fertility is suggested. Reduction in total number of pregnancies as well as size of litters produced (Table I) makes possible both prevention of fertilization and early embryonic death. There was no evidence of increased implantation sites or reabsorptions in treated mice.

Spermatogenesis in mice is apparently more

TABLE II. Per Cent Motility and Density of Spermatozoa from Male Mice Treated with Triethylenemelamine.*

	Spermatozoa	Treated Avg	Treated Range	Untreated Avg	Untreated Range
% motility†	Ductus deferentes	58	20-70	56	25-70
	Cauda epididymides	51	20-70	52	20-70
Density‡	Ductus deferentes	11.2	6-16	10.9	7-14
	Cauda epididymides	13.3	7-23	13.1	10-17

* Inj. intraper. with 0.2 mg/kg for each of 5 consecutive days.
† Data from 44 treated and 49 untreated male mice.
‡ Data from 10 treated and 10 untreated male mice in millions of cells/cc.

sensitive to x-rays than in rats, as reflected by induced oligospermia with the same low dose (9,10). Since TEM is a radiomimetic drug (1,2), it is surprising that, unlike the rat(2), mice treated with the same dose did not show structural defects in spermatogenesis. However, our interval betwen TEM treatment and fixation of testes may not have been long enough for manifestation of changes.

Summary. Triethylenemelamine (TEM) has been shown to affect adversely the reproductive capacity of male mice by acting on and through spermatozoa without altering their number, motility, or appearance. Females artificially inseminated with spermatozoa from the ductus deferentes and cauda epididymides of treated males showed a marked reduction in per cent fertility as well as litter size. Spermatozoa appeared more sensitive to TEM than late spermatids. No obvious structural changes were induced in testicular elements during treatment. The motility of spermatozoa was unchanged by exposure to a high concentration of TEM *in vitro*.

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Pyridoxal Phosphate in Plasma and Leukocytes of Normal and Pregnant Subjects Following B_6 Load Tests.* (25515)

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The concentration of pyridoxal phosphate, the coenzymatically available active form of Vit. B_6, has been estimated in leukocytes of normal subjects(1,2), and in those of pregnant women and cord blood(2), using a modification of the manometric method of Umbreit *et al.*(3). Boxer(1) also tried to utilize the method for determination of pyridoxal phosphate in total blood, but did not find measurable quantities in most human bloods unless the subjects were given oral doses of Vit. B_6. Comparisons of plasma and leukocyte values are herein described. Normal subjects, pregnant women and cord blood of newborn babies were studied. In addition, load tests were performed in normal controls, in a group in last trimester of pregnancy and in women who delivered their babies one day prior to test. We investigated whether load tests would give information not readily obtainable by single plasma or leukocyte determinations.

Method. Whole blood contains interfering substances necessitating use of diluted blood (1). Preliminary experiments were, therefore, undertaken to establish optimal dilution for performance of test. Plasma diluted 1:6, 82% of added Vit. B_6 could be recovered, whether or not its initial content was normal or relatively large, *e.g.*, obtained from individuals who had ingested a large amount of Vit. B_6. In contrast, in whole blood diluted 1:6, only 68%, and in packed red cells, only 61% of added pyridoxal phosphate could be recovered (Table I). For simultaneous estimation of the vitamin in leukocytes and plasma, 5 ml

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