Possible Mechanism of Posttesticular Antifertility Action of 3-Chloro-1,2-propanediol[1] (34281)

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The startling report of Ericsson (1) that 3-chloro-1,2-propanediol (U-5897, Upjohn) caused sterility in male rats without diminishing spermatogenesis or libido prompted these investigations on its possible mode of action.

Materials. Animals. Mature CD male rats (Charles River Breeding Laboratories, Inc.) were used to test the effects of 3-chloro-1,2-propanediol; the age and size of animal is indicated below for each experimental group. Female CD rats, 14–20 wks of age, (250–350 g) were used only to test the fertility of males. In the initial breeding studies all females were virgin; in subsequent experiments the females used were either nulliparous or uniparous, being equally distributed within the various experimental groups.

3-Chloro-1,2-propanediol. For the first series of experiments the Upjohn preparation, U-5897, was used, 98% solution of 3-chloro-1,2-propanediol in 0.025% aqueous methylcellulose (2); in all other studies 3-chloro-1,2-propanediol was purchased from K & K Laboratories, Inc. and diluted with methylcellulose accordingly. The two preparations gave identical results. These stock solutions contained 1.3 g/ml of 3-chloro-1,-2-propanediol (sp gr 1.326). Working solutions were prepared once weekly by diluting the stock solution 1:100 with distilled water; the working solution (13 mg/ml) was kept refrigerated and was used for daily oral administration to males (by stomach tube) in volumes of 0.20–0.69 ml, depending on the size of rat and dose.

Experimental groups. Rats used in Fertility tests. Thirty-four 14-week-old male rats (400 g), 4 groups of 8–10 rats each, were given 3-chloro-1,2-propanediol in doses of 6.5, 9.5, 12.5, or 15 mg/kg daily. Twenty other males of the same age and body size, given water or glycerol, served as controls. All rats received this treatment every a.m. for 14 days. At 4 p.m. after day 10 of administration, each male was housed in a cage with 5 females. They were left together for 5 nights. Vaginal smears were taken on all females each a.m.; after staining by the Papanicolaou technique, smears were examined for the presence of spermatozoa to determine whether mating had occurred. At 8 a.m. after the fifth night the females were isolated for pregnancy observation and the males were killed for tissue studies (see below).

Rats not bred. (1) Varying administered dose. Fifty-two 10 to 14-week-old male rats (320–400 g), 4 groups of 8–30 rats each, were administered 3-chloro-1,2-propanediol in doses of 6.5, 9.5, 12.5, or 15 mg/kg daily. Thirty-eight other males of the same age, given water, served as controls. All rats were killed for tissue studies on day 10 after 9 administrations of drug (or water), their status being comparable to that of rats used in fertility studies at the initiation of breeding tests.

(2) Varying time of administration. Thirty-three 20-week-old rats (av 520 g), 11 groups of 3 rats each, were given 3-chloro-1,2-propanediol, 15 mg/kg daily, in consecutive administration from 1 to 10 or 14 days. A comparable number of rats of the same age and body size was given water or glycerol as controls. All rats were killed for tissue studies the day after their last treatment.

(3) Surgical animals. Two other groups of rats received daily administration of 15

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TABLE I. Effect of 3-Chloro-1,2-propanediol (6.5–15 mg/kg daily) on Fertility of Male Rats from Days 10–14 of Treatment.

Treatment	Dose (mg/kg)	No. fertile /no. bred	Fertility (%)
Water	—	9/10	90
Glycerol	—	8/10	80
3-Chloro-1,2-propanediol	6.5	0/8	0
	9.5	0/8	0
	12.5	0/8	0
	15.0	0/10	0

mg/kg of 3-chloro-1,2-propanediol (or water) for 9 days and were killed the following day. These were (a) 60 rats which had been subjected to surgery involving severance of the vasa efferentia on one side 4 weeks prior to drug administration, and (b) 24 rats which had been rendered unilaterally cryptorchid 22 weeks prior to drug treatment. At the time of the first drug administration the rats in (a) were 14 weeks of age (av 400 g), and in (b) were 34 weeks of age (av 600 g).

Tissue studies. When males from the above experimental groups were killed, the following tissues were dissected, examined grossly, weighed, fixed in Bouin's fluid, and processed for microscopic study: testis, epididymis (caput, corpus, cauda), vas deferens, dorsolateral prostate, ventral prostate, seminal vesicles, and coagulating gland.

Results. Fertility records. Rats which received 6.5, 95., 12.5, or 15 mg/kg daily for 14 days were infertile when tested from days 10–14 (Table I). Reproductive failure was not because of diminished libido since the percentage of males which mated with females in the drug-treated group was comparable to that found in the control group (Table II).

Tissue weights. Treatment (15) mg/kg. In some rats, even after 1 or 2 administrations of 3-chloro-1,2-propanediol, it was obvious that the proximal caput and epididymal fat pad were edematous. The distended appearance of the epididymis became more obvious as treatment was continued. Table III reveals

TABLE III. Effect of 3-Chloro-1,2-propanediol (9-day treatment, 15 mg/kg daily) on Weights of Reproductive Tissues in Male Rats.

Organ (one side)	Wet wt (mg)[a]		Change (%) over control[b,c]
	Water control	3-Chloro-1,2-propanediol	
Testis	1666	1832	—
Caput epid.	222	313	+41.0
Corpus epid.	72	95	+31.9
Cauda epid.	201	235	+16.9
Vas deferens	94	93	—
Dorsolat. pros.	211	212	—
Ventral pros.	251	243	—
Sem. vesicles	521	500	—
Coag. gland	82	77	—

[a] Mean values for 30 rats.

[b] Comparable changes were noted when tissues were dried before weighing.

[c] Listed only if statistically significant ($p<0.01$).

that after 9 days of administration the caput, corpus, and cauda were 41, 32, and 17% heavier than controls; there were no significant alterations in weights of other tissues of the male reproductive tract. Comparable results were found in males which were bred and killed after 14 days of treatment.

Treatment (6.5–12.5 mg/kg). Table IV indicates that there were no alterations in weight of epididymis after 9 days of treatment with the 6.5 mg/kg dose of 3-chloro-1,2-propanediol. With 9.5 mg/kg the caput showed a small but significant increase; with the 12.5 mg/kg dose the caput and corpus were 41 and 19% heavier. Comparable re-

TABLE II. Effect of 3-Chloro-1,2-propanediol (15 mg/kg daily) on Mating Reaction of Male Rats from Days 10–14 of Treatment.

Treatment	No. of males	No. of females with sperm/total no. of females	Mated (%)	No. of females with litters/no. of females with sperm	Fertile (%)
Water	10	31/50	62	22/31	71
3-Chloro-1,2-propanediol	10	28/50	56	0/28	0

TABLE IV. Effect of Varying Doses of 3-Chloro-1,2-propanediol (9-day treatment, 6.5–12.5 mg/kg daily) on Weights of Epididymis in Male Rats.

		Treatment dose of 3-chloro-1,2-propanediol (mg/kg):					
		6.5		9.5		12.5	
Epididymis (one side)	Water control (mg)[a]	(mg)[a]	Change (%) over control[b]	(mg)[a]	Change (%) over control[b]	(mg)[a]	Change (%) over control[b]
Caput	189	195	—	208	+10.5	266	+41.1
Corpus	56	57	—	62	—	67	+19.3
Cauda	160	163	—	164	—	172	—

[a] Mean values for 8 rats.

[b] Listed only if statistically significant ($p < 0.01$).

sults were found in males which were bred and killed after 14 days of treatment.

Treatment in surgical rats. Table V shows that treatment (9 days) with 15 mg/kg of 3-chloro-1,2-propanediol produced no changes in weight of the epididymal tract on the surgical side of rats which had the vasa efferentia severed or had been subjected to cryptorchidy. The nonoperated side, however, showed the characteristic weight increases.

Microscopic examination. The earliest pathological change which was detectable after administration of 15 mg/kg of 3-chloro-1, 2-propanediol was peritubular edema confined to the initial segment of the caput. Some rats showed this effect even after a single treatment. After 4–14 days at this dose all rats, whether bred or not, showed intratubular dilatation of varying degrees in all zones of the epididymis; frequently this was most prominant in the initial segment of the caput. Some rats showed spermatocele formation in the initial segment. The vasa efferentia were not initially involved; dilatation and spermatoceles of the efferent ducts became apparent only in those rats in which there was severe enough involvement of the initial segment to cause back-up pathology involving the vasa efferentia and testes secondarily. At no time was there evidence of hemorrhage. The blood vessels of the internal spermatic artery–pampiniform plexus complex and the superior epididymal branches nourishing the proximal caput showed no unusual changes; however, the lymphatics always appeared dilated.

There were no pathological changes evident in the epididymal tract of rats, bred or

TABLE V. Effect of 3-Chloro-1,2-propanediol (9-day treatment, 15 mg/kg daily) on Weights of Epididymides in Male Rats with Unilateral Surgery.

	Intact side			Surgical side		
Epididymis	Water control	3-Chloro-1,2-propanediol	Change (%) over control[a]	Water control	3-Chloro-1,2-propanediol	Change (%) over control[a]
Vasa efferentia severance[b]						
Caput	222	313	+41.0	136	121	—
Corpus	72	95	+31.9	46	43	—
Cauda	201	235	+16.9	118	119	—
Cryptorchidy[c]						
Caput	231	360	+55.8	73	77	—
Corpus	62	78	+25.9	38	34	—
Cauda	253	276	+9.1	105	102	—

[a] Listed only if statistically significant ($p < 0.01$).

[b] Mean values for 30 rats (water control) and 30 rats (drug-treated).

[c] Mean values for 8 rats (water control) and 16 rats (drug-treated).

not bred, which received 9–14 days of treatment with 6.5 mg/kg of 3-chloro-1,-2-propanediol. With the 9.5 mg/kg dose there was a minimal degree of peritubular edema in the initial segment with some intratubular dilatation throughout the remainder of the epididymal tract. The degree of pathological involvement with 12.5 mg/kg approached that already described for 15 mg/kg.

The epididymides of rats in which (a) the vasa efferentia had been severed unilaterally, or (b) one testis was surgically cryptorchid were characteristically contracted, being devoid of spermatozoa and testicular fluid. In contrast to the nonoperated side which showed the characteristic drug-induced peritubular edema and intratubular dilatation, the surgical side showed no reaction from 9 days of treatment with 15 mg/kg of 3-chloro-1,2-propanediol.

Discussion. The posttesticular antifertility action of 3-chloro-1,2-propanediol is apparently not related to excessively high concentrations of this compound in seminal fluid, nor is it firmly bound to spermatozoa (3). When reproductive tissues of male rats are weighed and examined microscopically following treatment with a low antifertility dose, 6.5 mg/kg, the mechanism still remains obscure. But the increased weights, peritubular edema and intratubular dilatation of the epididymis following the administration of higher doses, beginning with 9.5 mg/kg, suggests an interference with absorption of testicular fluid and/or secretory products of the epididymis which may be necessary for the final maturation of spermatozoa. It is not inconceivable that some subtle effect of malabsorption may be in progress at lower doses. The fact that there is no response to this drug by epididymal tissues when the tract is empty, such as following severance of the vasa efferentia or cryptorchidy, indicates that the presence of luminal fluid may be essential to activate the mechanism of absorption upon which this drug acts. The actual metabolic pathway in the epididymis which is vulnerable to 3-chloro-1,2-propanediol has not yet been revealed. It is tempting, however, to consider that this drug may act as a toxic form of glycerol, interfering with the proper synthesis or utilization of one of the main secretory products of the epididymis, glycerylphosphorylcholine (4).

Summary. Oral administration of 3-chloro-1,2-propanediol (6.5–15 mg/kg daily) produces an antifertility effect in males without interfering with spermatogenesis or diminishing libido. With the lowest effective dose tested, 6.5 mg/kg daily, there is no histological evidence by 14 days which reveals a mechanism of action. With slightly higher doses, beginning with 9.5 mg/kg, peritubular edema, intratubular dilatation and increased tissue weights, beginning in the initial segment of the caput, suggests an interference with absorptive capacities of the epididymis.

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