Effects of Antifertility Agents on Male Mice as Determined by a Serial Mating Method (33948)

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There has been considerable experimental work on the conrol of fertility in the male (1–3) since the survey by Nelson (4) discussing those points in the male reproductive process most vulnerable to interference, namely, reduction division of primary spermatocytes, formation of sperm from spermatids, physiological maturation of sperm in the epididymis, and formation of seminal fluid. Histological evaluation of drug effects upon the testis is a valid criterion for complementary assessment of antifertility activity, but it is a laborious procedure for preliminary testing or screening of new drugs. In addition, it is now known that antifertility effects may be produced without apparent structural damage in testicular cells (3). Therefore, breeding experiments are necessary to accurately assess fertility.

Spermatogenesis is continuous in rodents, and proceeds at a constant rate, irrespective of sexual activity. Spermatogonia appear in the ejaculate as mature sperm in 56–63 days in rats and in 35–42 days in mice (3). In the serial or successive mating technique, different groups of normal females are exposed to treated males at weekly intervals during and for several weeks following the treatment period. The successive matings insure that germ cells present in all the various stages of development at the time of drug treatment are "sampled" and tested, and drugs affecting any stage of spermatogenesis are detected even with a relatively short treatment period (2). The cohabitation method in which the males are pretreated with drug before mating to a single group of females (5), however, would require pretreatment for 6 or more weeks to insure comparable detection of drug effects at all stages of spermatogenesis.

Rats have been the animals most commonly used for detecting antifertility activity of drugs, although mice have a shorter cycle of spermatogenesis. Since Jackson has suggested that the successive mating technique could be used in mice as well as rats (3), the applicability of mice for antifertility testing was investigated in the present work.

Materials and Methods. White Swiss mice were obtained from Simonsen Laboratories (Gilroy, Calif.). They weighed 27–30 g at 8–9 weeks of age and were acclimated to the laboratory for at least 1 week before being used. The mice were housed in a quiet room maintained at a temperature of 72–76°F, with lighting regulated on a schedule of 12 hr light and 12 hr dark. Humidity was not controlled but was monitored continuously and found to remain between 45 and 55%.

The males were treated with drug or vehicle orally for 3 weeks. Drugs were administered daily in 0.1 ml of olive oil, and fresh solutions or suspensions were prepared each week.

Serial matings were employed, starting at the beginning of the third week of drug treatment. Each male was caged with two different females on weeks 3, 4, 5, and 6 of the test. Thus, a total of 8 females was exposed to each drug-treated or control male. The females were autopsied 10 days after the mating period, and the number of viable fetuses was recorded. A female with one or more viable fetuses was defined as pregnant.

The drugs used were Thiotepa (*N, N′ N″* triethylenethiophosphoramide), nitrofurazone (5-nitro-2-furaldehyde semicarbazone), Win 18,446 or *N, N′*-bis(dichloracetyl)-1,8- octanediamine, and nafoxadine HCl or 1-{2-[*p*-(3,4-dihydro-6-methoxy-2-phenyl-1-naphtyl) phenoxy]ethyl} pyrrolidine hydrochloride.

Results. Figure 1 shows dose response curves for Thiotepa, nitrofurazone, Win 18,446 and nafoxadine, based on the percentage of pregnancies in all females exposed to the treated males during weeks 3 through 6 of the test. The pregnancy rate for the control mice ranged from 68 to 96%, with most con-

TABLE I. Effects of Thiotepa, Nitrofurazone, Win 18,446, and Nafoxadine on Fertility of Male Mice.

Drug	Oral dose (mg/day)	No. of male mice treated	Week no.:	Viable fetuses/female (mean ± SE) 3	4	5	6
Thiotepa	0.5	3		0[b]	0[b]	0[b]	0
Control	—	3		13.3 ± 0.8	11.8 ± 0.3	9.2 ± 2.0	5.8 ± 2.6
Thiotepa	0.1	6		0[b]	0[b]	0[b]	0[b]
Control	—	12		9.2 ± 1.0	7.8 ± 1.0	8.5 ± 0.8	9.6 ± 0.8
Thiotepa	0.05	3		0[b]	0.2 ± 0.2[b]	3.5 ± 1.5[b]	6.0 ± 2.0[b]
Control	—	9		7.8 ± 1.2	6.4 ± 1.2	8.3 ± 0.8	10.9 ± 0.5
Thiotepa	0.02	3		1.0 ± 0.8[b]	2.0 ± 1.3[b]	5.2 ± 1.3	4.0 ± 2.6
Control	—	3		13.3 ± 0.8	11.8 ± 6.3	9.2 ± 2.0	5.8 ± 2.6
Nitrofurazone	6	3		0[b]	0[b]	0[b]	0
Control	—	3		13.3 ± 0.8	11.8 ± 0.3	9.2 ± 2.0	5.8 ± 2.6
Nitrofurazone	3	6		2.3 ± 1.3[b]	0.1 ± 0.1[b]	0[b]	0[b]
Control	—	12		9.2 ± 1.0	7.8 ± 1.0	8.5 ± 0.8	9.6 ± 0.8
Nitrofurazone	2	6		1.7 ± 0.9[b]	0[b]	0[b]	2.5 ± 0.4[b]
Control	—	12		8.0 ± 0.9	7.1 ± 0.9	8.4 ± 0.8	10.7 ± 0.4
Nitrofurazone	1.5	6		8.4 ± 1.6	6.5 ± 1.4	5.3 ± 1.8	6.5 ± 1.8
Control	—	12		9.2 ± 1.0	7.8 ± 1.0	8.5 ± 0.8	9.6 ± 0.8
Nitrofurazone	1	3		7.2 ± 1.5	4.7 ± 2.1	10.3 ± 1.1	10.2 ± 1.0
Control	—	3		8.8 ± 1.0	9.3 ± 0.6	8.7 ± 1.8	10.2 ± 1.0
Nitrofurazone	0.75	3		4.8 ± 2.2[b]	12.7 ± 0.7	10.2 ± 0.9	10.0 ± 2.1
Control	—	3		13.3 ± 0.8	11.8 ± 0.3	9.2 ± 2.0	5.8 ± 2.6
Win 18,446	6	6		4.9 ± 1.4[b]	3.8 ± 1.6[b]	2.1 ± 1.0[b]	1.0 ± 0.8[b]
Control	—	6		11.1 ± 0.9	10.6 ± 0.5	8.9 ± 1.3	8.0 ± 1.5
Win 18,446	3	6		5.1 ± 1.6	2.7 ± 1.4[b]	4.1 ± 1.2	5.2 ± 1.6[a]
Control	—	9		8.6 ± 1.3	8.0 ± 1.2	6.8 ± 1.4	9.5 ± 1.3
Win 18,446	1	3		9.3 ± 0.8	9.7 ± 2.0	6.2 ± 2.2	7.2 ± 2.6
Control	—	6		6.2 ± 1.5	6.1 ± 1.6	5.6 ± 1.7	11.3 ± 1.2

[a] $p < 0.05$.
[b] $p < 0.01$.

trol groups having an average pregnancy rate of 80–90%. Of the drugs tested, Thiotepa was most potent, followed by nitrofurazone and Win 18,446. Nafoxidine was inactive. The average number of viable fetuses/female for each period of weekly exposure to the males is shown in Table I. Thiotepa was active at doses of 0.5, 0.1, and 0.05 mg/day, and induced a significant reduction in number of fetuses during weeks 3 and 4 with administration of 0.02 mg/day. The administration of nitrofurazone at doses of 6, 3, and 2 mg/day reduced the number of viable fetuses during all weeks of exposure, whereas

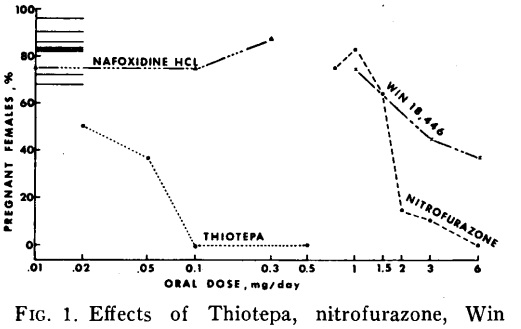

FIG. 1. Effects of Thiotepa, nitrofurazone, Win 18,446 and nafoxadine on fertility of male mice: combined pregnancy rate of females exposed to treated males on weeks 3, 4, 5, and 6 of experiment; solid lines represent mean control values.

lower doses did not. Treatment with Win 18,446 at 6 mg/day reduced the number of viable fetuses significantly for all weeks of exposure; at 3 mg/day some reduction in the number of fetuses was noted during weeks 4 and 6, and the 1 mg/day dose was inactive.

Discussion. These experiments indicate that the antifertility activity of Thiotepa, nitrofurazone and Win 18,446 can be demonstrated in male mice with the experimental procedure described. Nafoxadine was inactive in this test, although it has been reported to have antifertility effects in male rabbits, rats, and dogs treated chronically (6). Nafoxadine is an estrogen, and other estrogens are known to cause interference with spermatogenesis in mice as well as rats (7). Possibly, nafoxadine would have antifertility effects in male mice if it were administered for a longer period of time.

The successive mating technique has been used by Jackson to help delineate the cell stage of spermatogenesis affected by short courses of drug treatment in rats (8). However, even with short treatment schedules, it is difficult to pinpoint a single stage at which a drug acts because rats show considerable variation in fertility from week to week (9), and some drugs have multiple and cumulative effects (3).

The method employed in the present experiments represents a compromise that may offer a number of advantages for drug screening. By treating the mice for 2 weeks before mating is begun, the method detects drugs affecting formation of seminal fluid, maturation and function of spermatozoa, and all stages of spermatogenesis. In antifertility screening it may be most desirable to detect drugs affecting postmeiotic differentiation of germ cells, whereas in toxicological studies, it is important to detect drugs acting at meiotic and premeiotic stages of spermatogenesis as well. Using mice and the serial mating technique reduce the amount of drug required for antifertility testing. Drug requirement could be reduced further by decreasing the length of the treatment period, but the advantage gained must be balanced against the risk of not detecting active compounds. In addition, mice have a shorter spermatogenesis cycle than rats (3), which means that fewer sets of females are required for serial matings. Male and female mice of proven fertility were not used in these experiments because the fertility rates in the control mice were satisfactory for a screening test. The practical advantages offered for the use of mice, and the activity demonstrated for known antifertility agents in this test procedure suggest that this method may be useful for drug screening.

Summary. A method employing male mice and a serial mating technique was described as a screening test for assessing the antifertility effects of drugs in the male. Thiotepa, nitrofurazone, and Win 18,446 were active in this test while nafoxadine was inactive.

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