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Deuterium oxide in the drinking water of either male or female mice produces sterility. Male mice are more sensitive to the effects of D_2O than are female mice, and C_{57} mice appear to be more sensitive than Swiss mice.[1]

We have investigated further some of the conditions--with particular reference to time--of D_2O treatment required to produce sterile C_{57} male mice. These experiments indicate that the sensitive phase of sperm production is probably centered around the late prophase of meiosis.

EXPERIMENTAL PROCEDURES AND RESULTS

Production of Sterility -- In order to determine the minimum time required to produce sterility in C_{57} male mice by the substitution of D_2O in the drinking water for ordinary water, 10 male mice maintained on 30% D_2O were individually mated for 1-week intervals with C_{57} female mice. The female mice were maintained on 30% D_2O only for the 1-week period in which they were caged with a male mouse. At the end of each week, the females were replaced by additonal normal female mice, and the female mice that had been removed were caged individually to determine if pregnant. Offspring were counted two weeks after birth, and those pairs which did not produce a litter during a 28-day period after the initial mating were considered to be sterile. The results summarized in Table I indicate that all male mice were sterile 28 days after initiation of D_2O treatment. The male mice may have been sterile as soon as 23 days after the initiation of D_2O treatment, since eight of the pairs that were first mated at 21 days produced litters 20 to 22 days after the start of mating.

In order to determine if treatment with D_2O for a relatively short time would produce sterility in mice subsequent to withdrawal of the D_2O groups of 10 C_{57} male mice were given 30% D_2O for periods of 1, 2, and 3 weeks and individually mated for 1-week intervals with C_{57} female mice. No subsequent sterility was observed in the group of 10 mice treated with D_2O for 1 week. The mice treated for 2 weeks with 30% D_2O were not sterile at the end of the 2-week treatment or 1 week later, but they were sterile 4 weeks after the initiation of treatment, i.e., 2 weeks after D_2O treatment had been discontinued. (Seven pairs produced no litters and litters from the other three died. Five of the 10 mice mated were sterile 35 days from the initiation of treatment or 21 days after D_2O treatment had been terminated and the offspring from two other litters died (Table II.) The three viable litters were born 26 days after the initial mating. The mice treated for 3 weeks were also sterile 4 weeks after initiation of D_2O treatment, and some sterility was indicated 28 days after D_2O was removed. The female mice that did not produce litters had not been given any D_2O in these experiments.

Effect of D_2O on Sperm -- To investigate the effects of D_2O treatment on the production and appearance of sperm in C_{57} mice, male mice were maintained on 30% D_2O in the drinking water for periods up to 11 weeks. At weekly intervals, two or more experimental mice and at least one control mouse were sacrificed and the right and left vas deferens were removed and separately rinsed with 1 ml of 0.9% NaCl. The presence or absence of motile sperm was first determined. After the addition of 1 drop of Ehrlich's hematoxylin, the sperm were counted in a blood counting chamber and the sperm per vas deferens was calculated. No consistent effect was observed on the number of sperm present until 10 to 11 weeks of treatment, after which time most of the sperm counts had dropped to 10 or less of normal. However, after 4 weeks of treatment with D_2O a considerable number of broken and nonmotile sperm were observed. Broken sperm were not included in the sperm count.

The testes and epididymes were removed and prepared for histological studies. Preliminary examination of sections stained with hematoxylin and eosin and with periodic acid-Schiff reagent (PAS) have indicated degenerative changes in some tubules as early as 2 weeks after D_2O administration. Further studies are in progress.

DISCUSSION

Our experiments and those of others have shown that the sensitivity of biological systems to D_2O varies considerably. It has been reported that some bacteria show no effects until deuterium concentrations in the medium exceed 90% (2). Chlorella fail to divide and their growth is inhibited when the D_2O concentration in the medium exceeds 70%; however, by serial subculture, Chlorella can be adapted to grow nearly normally at D_2O concentrations of 60 to 70% (3).

Fifty per cent D_2O in the drinking water is fatal to mice within a few days; 40% D_2O in the drinking water for several months produces gross physiological effects such as emaciation, epilation, and blindness. No obvious adverse effects, except sterility, are produced by 30% D_2O even after 4 months. Katz et al. (2, 4) have shown that the body-fluid deuteration reaches an equilibrium value of about 24% D_2O in about 6 days when 30% D_2O is administered, and the mouse tissues reach a maximum deuterium content equal to 40% of that of the tissue fluids. Thus, sterility in male mice is produced when less than 10 atom per cent excess deuterium is in the tissue. Some phase of the spermatogenic process would appear to be among the most sensitive known biological systems to the effects of D_2O.

Sperm development is a continuous process; about 40 days is required to obtain ejaculated sperm from spermatogonia (5). The data presented in Table I indicate that mice became sterile between 23 and 28 days after the initiation of D_2O treatment. The body fluids attain about 70% of their equilibrium concentration within 2 days, and full equilibrium concentration in about 6 days. Therefore, the viability of mature sperm is not affected merely by the presence of D_2O in the tissue fluids. The experiments summarized in Table II indicate that sterility can be produced in mice by administration of D_2O for as short a time as 2 weeks, but this sterility is not demonstrable until 4 weeks after initiation of D_2O treatment or 2 weeks after D_2O administration has been discontinued. It would appear that a particular phase of spermatogenic development is affected. It should be noted that sterile mice become fertile again about 4 weeks after D_2O is withdrawn.

The selective action of D_2O upon a particular stage of spermatogenesis can be compared to the action of other agents which have been reported by Jackson and co-workers (6, 7). Using rats, they found that triethylenemelamine interferes with a later stage of spermatogenesis, and about 6 weeks is required for recovery. However, it should be noted that sterility can be produced very quickly (within 1 week) with triethylenemelamine. Myleran (1, 4-dimethanesulfonoxybutane) produces no sterility until 8 weeks after a single dose, when sterility rapidly develops. Therefore, myleran is probably destroying spermatogonia but not partially developed sperm. The effect of radiation in rats is dose-dependent; 500r initially reduces litter size, and complete sterility is observed 7 weeks after irradiation. Thus all phases of spermatogenesis appear to be sensitive to radiation with the spermatogonia being completely destroyed.

Oakberg has presented a timetable of cell development in spermatogenesis of the mouse (5). In Figs. 1 and 2 we have superimposed the time of treatment with D_2O and its result upon a graphic representation of the timetable for mouse spermatogenesis. The darkened areas show the presence of D_2O during the indicated phases of sperm development. Thus, for mice treated 2 weeks with D_2O (Fig. 1) mature sperm and late spermatids developing in the presence of D_2O did not produce sterile matings, nor did sperm developing from spermatogonia and resting spermatocytes that had been in a D_2O environment exhibit sterility. The common phase of sperm development in which exposure to D_2O produced sterility includes the late prophase of the first meiosis and the early spermatids. The data from the experiment in which mice were treated 3 weeks with D_2O and mated for weekly intervals localize the principal effect of D_2O in the same developmental stage as obtained from the 2-week experiment. It is at this stage that chromosomal reorganization occurs. Hevesy has reported that aberrations, including a tendency to ball together in irregular clumps, were observed in the chromosomes of the intestines of mice administered 30% D_2O (8).

The sperm of mice given 30% D_2O did not show any gross morphological changes until 4 weeks of treatment with D_2O. At this time, numerous sperm were observed that had fragmented, and many nonmotile sperm were also seen. After longer periods of D_2O treatment, an increasingly large number of sperm were nonmotile. However, some sperm from mice known to be sterile appeared normal when observed under low-power (70x) magnification. The production of sperm appeared to be nearly completely inhibited only after extended treatment (10 or more weeks) with 30% D_2O.

As yet, the mechanism of the production of sterility by D_2O has not been clearly shown. Possibly, the summation of the known small effects of deuterium on enzymic rates i reaction may produce metabolic imbalances culminating in death of the organism. However, the observation that late spermatids and mature sperm are apparently not damaged by D_2O suggests that this is not a major effect.

On the other hand, the appearance of sterility in male mice some 2 weeks or more after their return to normal drinking water indicates that the effect of D_2O was impressed upon information-carrying units in developing sperm. Although D_2O was almost completely absent during the maturation of the sperm and when the mice mated (9), the sperm were still able to exhibit the effects of their much earlier contact with D_2O in a variety of ways. Some of the offspring from these matings reached parturition but did not live, others died during gestation, and presumably still another phase of embryonal development was affected. The appearance of aberrations [illegible]

sections of the testes suggests that these effects could make themselves apparent in the developing sperm itself. One can hardly escape the conclusion that the presence of D_2O during the late prophase and first meiotic division (possibly the early spermatid as well) interfered with the normal construction of the genetic material itself. Such abnormalities would then manifest themselves all along the developmental sequence, depending on their nature and number.

The formation of giant algal and ascites tumor cells (3, 4) and the production of sterility in mice suggests strongly that the mechanism is at the macromolecular level, quite possibly due to changes in the physical-chemical forces binding macromolecules--such as protein and nucleic acids--in which important structural features are maintained by the participation of many hydrogen bonds (10, 11). A change of this hydrogen bonding will affect the specificity of the complementarity in the double helix of nucleic acid in the chromosomal structure and could lead to mutation in the sperm (12, 13). Further experiments in other biological systems are in progress to test this hypothesis. Direct observation is underway of the effect of deuterium on suitable physical and chemical properties of purines and pyrimidines as well as macromolecules (particularly polypeptides, proteins, enzymes, and nucleic acids).

SUMMARY

Some of the conditions required to produce sterility in male mice by D_2O have been investigated. Sterility can be produced in C_{57} male mice by substitution of 30% D_2O for normal water in the drinking water for 2 weeks. The mice become sterile 4 weeks after the initial treatment with D_2O, and in this experiment 2 weeks after normal water has again been provided in the drinking water. By this and other experiments the sensitive phase of sperm production has been shown to be centered around the late prophase of meiosis. It is suggested that the normal construction of the genetic material has been affected and that the mechanism of action of D_2O is at the macromolecular level.

Table I

TIME NECESSARY TO PRODUCE STERILE C_{57} MALE MICE BY 30% D_2O IN DRINKING WATER

Ten C_{57} male mice were given drinking water containing 30% D_2O instead of ordinary water. They were mated individually with female C_{57} mice for 1-week intervals. Offspring were counted 2 weeks after birth.

Time of Mating (Days after initiation of D_2O treatment)	Total Pregnancies	Total Viable Litters	Total Viable Offspring
7-13	9	7	37
14-20	6	6	32
21-27	8	7	41
28-34	0	0	0
35-41	0	0	0
42-48	0	0	0

Table II

PRODUCTION OF STERILITY IN C_{57} MALE MICE BY 30% D_2O IN DRINKING WATER

Ten C_{57} male mice were given drinking water containing 30% D_2O instead of ordinary water for 2 weeks or for 3 weeks. They were mated with female C_{57} mice for 1-week intervals. Offspring were counted 2 weeks after birth.

Time of Mating (Days after initial D_2O treatment)	Days After D_2O Treatment Discontinued	Total Pregnancies	Total Viable Litters	Total Viable Offspring
	TWO-WEEK TREATMENT			
0-6	- -	10	9	66
21-27	7-13	8	5	19
28-34	14-20	3	0	0
35-41	21-27	5	3	15
42-48	28-34	9	8	53
49-55	35-41	9	9	46
	THREE-WEEK TREATMENT			
0-6	---	9	9	52
28-34	7-13	1	0	0
35-41	14-20	2	1	3
42-48	21-27	5	5	20
49-55	28-34	6	5	37
56-62	35-41	10	9	46

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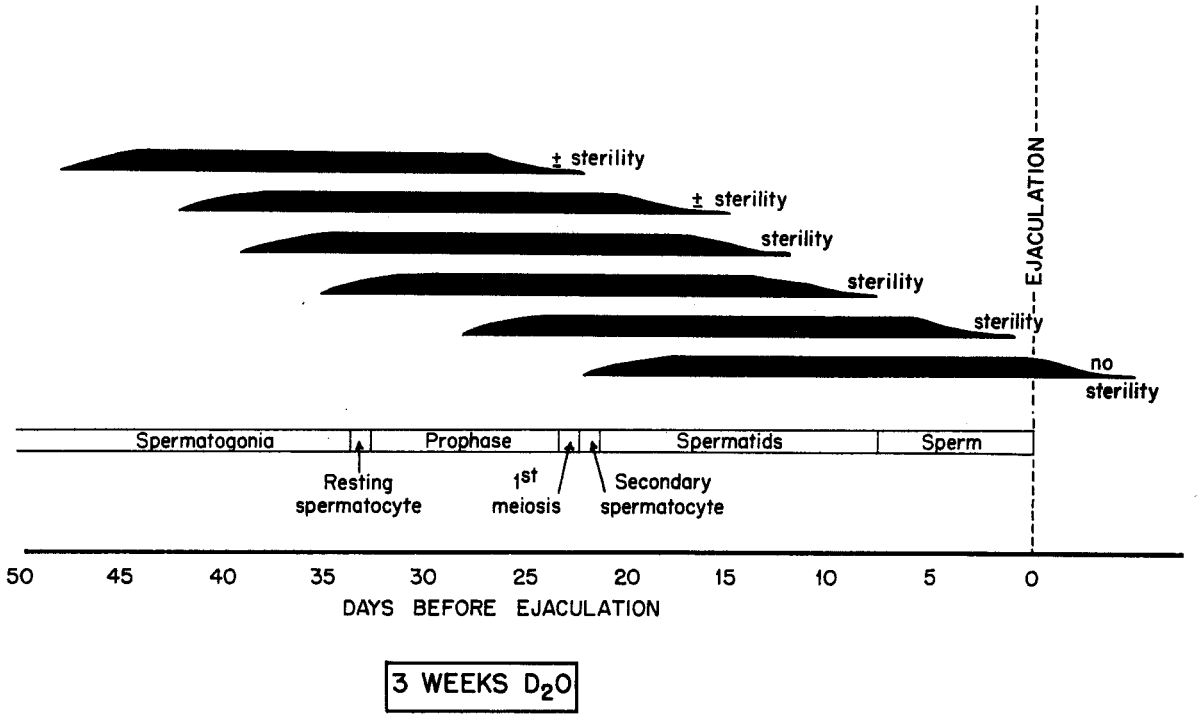

Fig. 1. A graphic representation of the effect of D_2O treatment for two weeks compared with the spermatogenic cycle.

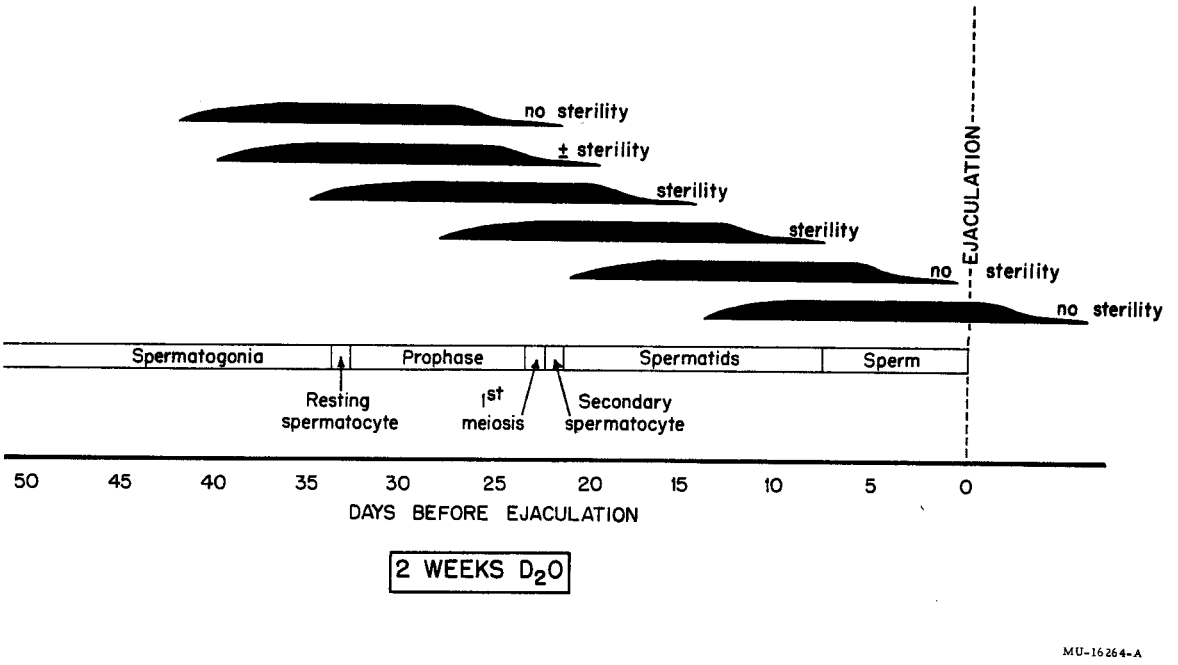

Fig. 2. A graphic representation of the effect of D_2O treatment for three weeks compared with the spermatogenic cycle.