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MALE MICE BY DEUTERIUM OXIDE

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FURTHER STUDIES ON STERILITY PRODUCED IN  
MALE MICE BY DEUTERIUM OXIDE

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Introduction

June 1960

We have previously reported that deuterium oxide in the drinking water of either male or female mice produces sterility.<sup>1,2,7</sup> An investigation of some of the conditions -- with particular reference to time -- of deuterium oxide treatment to produce sterile C<sub>57</sub> male mice indicated that the sensitive phase of sperm production centered around the late prophase of meiosis. In some experiments, although D<sub>2</sub>O was almost completely absent during maturation of the sperm, and when the mice mated, these sperm exhibited the effects of their much earlier contact with D<sub>2</sub>O. No viable offspring were obtained from these matings. We concluded that the presence of D<sub>2</sub>O during the late prophase and meiotic divisions interfered with the normal construction or division (or both) of genetic material. It was suggested that changes in the forces, principally hydrogen bonds, in macromolecules affected their structural characteristics and resulted in abnormal division.

The objective of the experiments reported here was to determine the phases of embryonic development of the mouse at which the lethal action of deuterium oxide on sperm is manifested. These investigations on embryonic growth initiated by sperm developed in D<sub>2</sub>O have yielded additional evidence that D<sub>2</sub>O severely damages the genetic material of developing sperm, with resulting sterility of the male mouse.

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<sup>7</sup> It is shown in this paper that the term "sterility" accurately connotes the effect in the male mouse. Czajka and Finkel<sup>3</sup> have suggested the term "loss of reproductive potential" to describe the effect of D<sub>2</sub>O upon the fertility of mice. This term is more descriptive of the effect of D<sub>2</sub>O in the female mouse.



No increase in the number of regressions was observed. (The high percentage of regressions obtained after 6 weeks of D<sub>2</sub>O treatment is due to one female mouse which had seven regressions.) After 2 weeks of treatment with D<sub>2</sub>O, no significant decrease in the percentage of pregnant females, or the litter size, was noted. However, beginning 3 weeks after administration of D<sub>2</sub>O a progressive decrease in both the percentage of pregnancies and the embryos per pregnancy was observed, until after 6 weeks of treatment with D<sub>2</sub>O, only 12% of the females became pregnant and the average number of embryos per pregnancy was 3.4. This compares with the over-all pregnancy ratio of 80% and 9.4 embryos per pregnancy observed in the control mice. Embryos per mating with the D<sub>2</sub>O-treated mice had dropped to 5% of that of the controls after 6 weeks (0.4 embryo per mating compared to 7.6 embryos per mating for the control).

Since each male mouse was mated with three (or four) female mice, the number of pregnant females obtained each week from each individual male can also be determined. This is shown in Table II. After 2 weeks of administration of D<sub>2</sub>O, 13 of the 15 experimental mice had either 2 or 3 fertile matings (out of 3 possible), and only one male mouse had no fertile matings (the following week he was fertile). This may be compared with the observations made at 6 weeks, when only two of the 15 males had 2 fertile matings (out of 4 possible) and ten of the males did not have a fertile mating.

The results of this experiment strongly suggest that sperm produced in the presence of D<sub>2</sub>O are either so defective that they are unable to penetrate the egg\* or that upon entering the egg fertilization occurs, but damaged genetic material leads to early death of the fertilized mouse egg.

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\* The presence of vaginal plugs indicates that copulation occurred by D<sub>2</sub>O-treated animals. Sperm motility is presumably not an important factor in the transport of sperm through the uterus and ovarian tubes.

Observations of Development of Mouse Eggs Fertilized by D<sub>2</sub>O-Treated Males

Eight male Swiss mice three months of age from the Lawrence Radiation Laboratory colony were provided with drinking water containing 30% D<sub>2</sub>O in lieu of tap water for 5-1/2 to 6 weeks. The male mice were placed at midnight in cages with virgin female mice in estrus. At 10:00 a.m. the following day, the females were removed and the presence of vaginal plugs determined. Thus the time of fertilization was known within 10 hours. Normal control mice were mated in the same fashion. At the desired time after copulation, the female mice were sacrificed, and the eggs flushed from each oviduct with 0.9% NaCl and observed under magnification up to 250 X after staining with acridine orange (1 to 40,000 in 0.9% NaCl and 0.01 M phosphate buffer, pH 7.4). The results are summarized in Table III.

In general, 55 to 65 hours after fertilization, most control mice had numerous eggs in the multicell stage (8 to 16 cells). Relatively few two- or four-cell eggs, or disintegrating eggs, were observed. However, a high frequency of abnormal eggs was obtained from females mated with D<sub>2</sub>O-treated mice; many were one, two, or four cells. The cells were often not of uniform size, and in many instances they were not congruent. A few of the aberrant cell types are shown in Fig. 1. These aberrant cells can be compared with examples of degenerating cells found in a study by Lewis and Wright.<sup>6</sup>

After the experimental males had been provided with 30% D<sub>2</sub>O for 7 weeks, they were returned to ordinary water. Two weeks later these mice were again mated with normal females, and the eggs were examined approximately 50 hours after copulation. Again, a high frequency of degenerating eggs was observed.

Discussion

A reduced number of litters and fewer mice per litter are obtained when male Swiss mice which have been maintained on 30% D<sub>2</sub>O in lieu of tap water are

mated with normal female Swiss mice. Nearly complete sterility is obtained when Swiss male mice are maintained on 30% D<sub>2</sub>O for 6 weeks. At this time, although many broken and nonmotile sperm are present in the vas deferens, 20,000 to 30,000 motile sperm were observed in each vas deferens of two sterile male mice which were examined, confirming a similar observation on C<sub>57</sub> mice.<sup>2</sup> It is extremely unlikely that the degeneration of the eggs could have resulted from the mere presence of 20% to 30% D<sub>2</sub>O in the sperm, since a sperm constitutes an extremely small fraction (much less than 0.1%) of the total mass of a fertilized egg. In addition, the gross sterility observations have shown that sterile matings result from male mice which have been returned to normal water for at least 2 weeks after a 2-week treatment with 30% D<sub>2</sub>O.<sup>2</sup> In the present study we found degenerating eggs when male mice that had been on D<sub>2</sub>O for 7 weeks and then returned to normal water for 2 weeks were mated with normal females. The sperm of mice returned to normal water for 2 weeks certainly have very little deuterium in them.

Our experiments have not shown that the sperm have entered the eggs. The observation of motile sperm would suggest that the sperm would be capable of entering the eggs. Such sperm, if abnormal in genetic complement, could lead to subsequent divisions which would be abnormal and which in many instances would not proceed past the one- to four-cell stage. Experiments to determine whether the sperm do enter the egg and whether fusion of the pronuclei does occur are in progress.

Our experiments indicate no increase in embryonic deaths after implantation. This is in marked contrast to the effects of radiation in which a large increase in such deaths, termed "dominant lethal mutation," occurs.<sup>4</sup> This suggests that the damage to the genetic material caused by D<sub>2</sub>O differs from damage caused by radiation.

Hermans, et al.<sup>7,8</sup> and Scheraga<sup>9</sup> have shown that the substitution of D for H in macromolecules affects the thermodynamics of the helix-coil transformation. With ribonuclease the temperature required for the helix-coil transition is raised by deuteration. Crespi et al.<sup>10</sup> have reported that elevated temperatures are necessary for growth of certain microorganisms in D<sub>2</sub>O, which is consistent with the hypothesis that the presence of D<sub>2</sub>O leads to an increased stability of the helix. Gross and Spindel<sup>11,12</sup> have shown that D<sub>2</sub>O causes a reversible inhibition of mitosis of Arbacia punctulata. In addition, they have demonstrated an increased viscosity of gelatin solutions as well as a higher liquefaction temperature in the presence of D<sub>2</sub>O.<sup>12</sup> Previous experiments indicated that the phase of sperm production most sensitive to D<sub>2</sub>O is the meiotic phase. Sperm that have undergone meiotic division in the presence of D<sub>2</sub>O during their genesis are not capable of producing offspring even though the animal is not subsequently supplied with D<sub>2</sub>O.

At this stage of sperm production, chromosomal reorganization occurs. It is suggested that the presence of deuterium markedly affects this process, principally by changes in the forces of association of macromolecules. Motile sperm are obtained, but the genetic material is so abnormal that although fertilization of the egg may occur, its death soon follows. Experiments are in progress to further characterize changes in the genetic material of sperm resulting from the presence of D<sub>2</sub>O.

Summary

Thirty percent D<sub>2</sub>O in the drinking water of male Swiss mice produces sterility. Both a decrease in litter size and a decreased percentage of fertile matings were observed when treated males were mated with normal females. No increase in regressions was obtained. The development of mouse eggs in a female mated with a D<sub>2</sub>O-treated male is generally abnormal; few multi-celled eggs are obtained 2 to 2 1/2 days after copulation. It is suggested that changes in physical forces that bind macromolecules are produced by the substitution of deuterium for hydrogen. These changes may result in abnormal genetic material in developing sperm.

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Table I  
Effect of 30% D<sub>2</sub>O in drinking water on fertility of male mice

	Time of treatment prior to mating (weeks)								Totals 2-to 6
	D <sub>2</sub> O-treated males (15)				Control males (10)				
	2	3	4	6*	2	3	4	6*	
<u>Mice with vaginal plugs</u>									
No. of females	24	23	23	17	16	11	11	8	46
No. pregnant	23	16	11	4	15	9	11	8	43
No. embryos	246	107	66	19	148	75	132	87	442
No. regressions	15	12	6	0	15	16	9	10	50
% regressions	5.7	10.1	8.3	0	9.2	17.6	6.4	10.3	10.2
Embryos/pregnancy	10.7	6.7	6.0	4.8	9.9	8.3	12.0	10.9	10.3
<u>Mice without vaginal plugs</u>									
No. of females	21	22	21	43	14	19	19	30	82
No. pregnant	11	12	6	3	10	15	12	23	60
No. embryos	84	76	42	5	77	138	119	197	531
No. regressions	11	12	7	8	12	15	6	19	52
% regressions	11.6	13.6	14.3	75	13.5	9.8	4.8	8.8	8.9
Embryos/pregnancy	7.6	6.3	7.0	1.7	7.7	9.2	9.9	8.6	8.9
<u>Combined</u>									
No. of females	45	45	44	60	30	30	30	38	128
Total No. pregnant	34	28	17	7	25	24	23	31	103
% pregnant	76	62	39	12	83.3	80	76.7	82	80
Total No. embryos	330	183	108	24	225	213	251	284	973
Total No. regressions	26	24	13	8	27	31	15	29	102
% regressions	7.3	11.6	10.7	25	10.7	12.7	5.6	9.3	9.5
Embryos/pregnancy	9.7	6.5	6.4	3.4	9.0	8.9	10.9	9.2	9.4
Embryos/mating	7.3	4.1	2.5	0.4	7.5	7.1	8.4	7.5	7.6

\* Four females/male

Table II

Frequency of fertile matings of  $D_2O$ -treated male and normal female mice \*

		No. of males associated with N pregnant females				
		Control male mice (10 total)				
Duration of Treatment (weeks)	No. pregnant females/male (N)					
		4	3	2	1	0
$H_2O$	2	-	5	5	0	0
	3	-	4	6	0	0
	4	-	4	5	1	0
	6	4	4	0	1	0
$D_2O$						
	$D_2O$ male mice (15 total)					
	2	-	7	6	1	1
	3	-	4	6	3	2
	4	-	2	5	2	6
	6	0	0	2	3	10

\* Three female mice were mated with each male except at 6 weeks, when 4 female mice were mated with each male.

Table III

Stage and condition of eggs from mice fertilized by normal or D<sub>2</sub>O-treated males

	No. females	Time after copulation (days)	Females without eggs	Condition of eggs	Stage of Eggs (No. of cells)					Total eggs
					1	2	3	4	8 or more	
Control males	5	1 1/2 to 2	1	N <sup>a</sup> D <sup>a</sup>	3 0	4 0	3 0	2 0	25 0	37 0
D <sub>2</sub> O treated males	3	1 1/2 to 2	0	N D	23 1	4 1	1 0	0 0	0 0	28 2
Control males	5	2 1/2 to 3	1	N D	0 1	0 1	0 0	0 0	28 0	28 2
D <sub>2</sub> O treated males	9	2 1/2 to 3	2	N D	9 1	4 6	0 2	0 1	4 33	17 43

a N = normal, D = degenerating

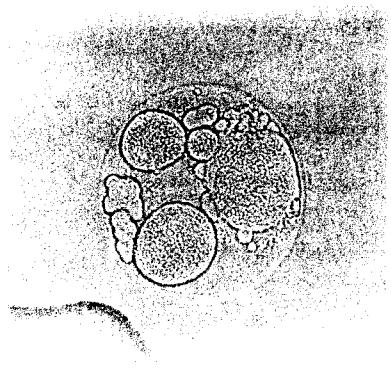
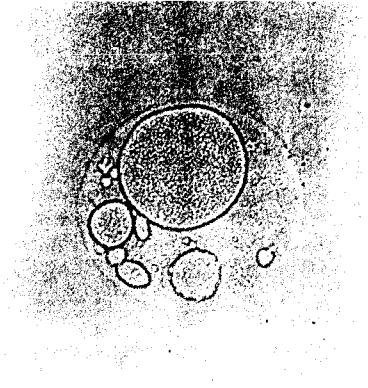
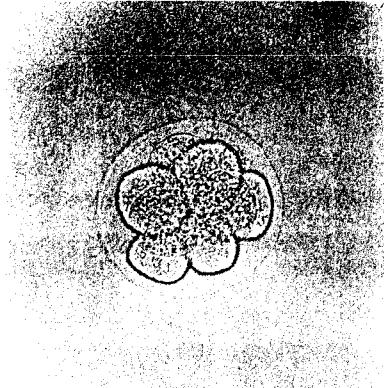


Fig. 1 Photomicrographs (X 250) of eggs obtained 2 to 2-1/2 days after copulation. Upper left is an example of a normal 8-cell egg. Upper right and both lower pictures are examples of abnormal cell types resulting from mating of  $D_2O$ -treated males with normal female mice.



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