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HISTOLOGICAL STUDIES ON THE MECHANISM OF D_2O-INDUCED STERILITY IN MICE

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Abstract

Male mice, given D_2O in their drinking water, are incapable of producing viable offspring. Eggs from females mated to such sterile males were examined using standard histological techniques. The data clearly demonstrate that the previously observed pre-implantation death is due to the failure of the sperm to fertilize the egg, not to subsequent abnormal cleavage of the fertilized egg.

In studies in which normal females were mated to D_2O-treated male mice, it has been shown that D_2O produces sterility in the males (1,2); however, the site of the damage has not been demonstrated. Since embryonic death after implantation did not increase in such females, cleavage stage embryos were examined 18 to 72 hours after mating (3). Most of the embryos present in the oviducts were either one-celled or, though "multicellular", were abnormal and degenerating; few normal embryos were found. These data did not indicate whether developmental failure was due to failure of the sperm to fertilize the egg or to a failure of the fertilized egg to develop. The experiment reported below utilized standard histological techniques to resolve this question.

C_{57} male mice from the University of California Cancer Research Laboratory, 10 to 14 weeks old at the beginning of the experiment, were used. This inbred strain has been shown to be more sensitive to the sterility-producing effects of D_2O than Swiss strain mice, which are

random bred (1). Because C_{57} males are known to have a high innate sterility, they were tested for fertility before the start of the experiment, and only those males which sired two viable litters were used. D_2O was given at the concentration of 30% by volume in the drinking water ad lib. At the end of four weeks' treatment, H_2O was given instead of D_2O as drinking water. Control males, mated weekly, were maintained under conditions identical to those of the experimental animals except that they drank H_2O throughout the experiment. All females were virgin Swiss mice, from Simonsen Laboratories, Gilroy, California.

Each male was caged with 8 (later 3) females nightly from 10 P.M. to 9 A.M. At four-hour intervals the females were examined for vaginal plugs. Thus the time of copulation could be estimated within four hours. As females were mated, they were removed and other females were placed in the cage. Females were sacrificed 12 to 16 hours after mating. Some mated females were allowed to go to term; their litters were counted at birth.

Oviducts were removed *in toto* and fixed in Bouin's. They were embedded in paraffin, serially sectioned at 10 μ, and stained with haematoxylin-eosin. All sections of each oviduct were examined with a light microscope; magnifications up to 640x were used. All slides were code-numbered, so that the experimenter did not know whether the eggs came from a control female or one mated to a treated male. Eggs were scored as "fertilized" if two pronuclei were observed or if a sperm tail and enlarging sperm head were visible in the ooplasm and/or if

the orientation, condensation and chromosome position in the oocyte meiotic spindle indicated that emission of the second polar body was in progress or complete. In eggs scored "unfertilized", an unchanged second meiotic spindle was present in the ooplasm and there was no evidence of sperm head or tail. Fragmented eggs and those in which the ooplasm showed unusual condensation, were scored as "degenerating".

The results are shown in Figure 1. By two weeks on D_2O, the average number of fertilized eggs had dropped from 5.8 per female (pooled data for females mated with control males and treated males at week zero) to 3.8 per female; after males had been on D_2O four weeks, no fertilized eggs were obtained from the mated females. A two-way analysis of variance was done on the data (4). The group treated with D_2O was significantly different from the control group ($F_{1,44} = 8.5$; $p \leq .01$). There was also a systematic change over the eight-week period ($F_{8,44} = 4.5$; $p \leq .01$), and this change was a function of whether the animals had been treated with D_2O—i.e., the interaction between treatment and time was significant ($F_{8,44} = 2.6$; $p \leq .05$). Table I contains the data on the number and size of litters obtained from those females which were allowed to go to term. From a control average of 7.3 viable offspring per female, there was a decrease to 0.3 offspring per female after two weeks' treatment and to no offspring after three weeks of D_2O administration to the males.

Under the conditions of this experiment, sperm penetration usually occurred within 6 to 8 hours after the females were removed from the males. Thus, by collecting eggs 12 hours after copulation, one could be sure that sperm entry would have been effected, yet most eggs should

not be old enough to start degenerating. The results presented here clearly indicate that the failure of the D_2O-treated males to produce viable offspring, as previously reported, was due to the failure of the sperm to fertilize the egg and not to a failure of the subsequentt development of the fertilized egg. These conclusions are supported by subsequent examination of 630 living eggs by phase contrast microscopy.

Histological study of the testes of D_2O-treated male mice is in progress; initial observations suggest that acrosome formation may be abnormal. However, until now, the mechanism of action of D_2O in producing sterility is not known. Autoradiographic studies of eggs from females mated to D_2O-treated males (in which the sperm are labelled isotopically) and electron microscopy of sperm from D_2O-treated males are also in progress in further attempts to pinpoint the site of action of D_2O.

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References and Notes

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4. We are indebted to Dr. Gordon Pryor of the Stanford Research Institute, Menlo Park, California, for the statistical analysis of the data.
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Table I

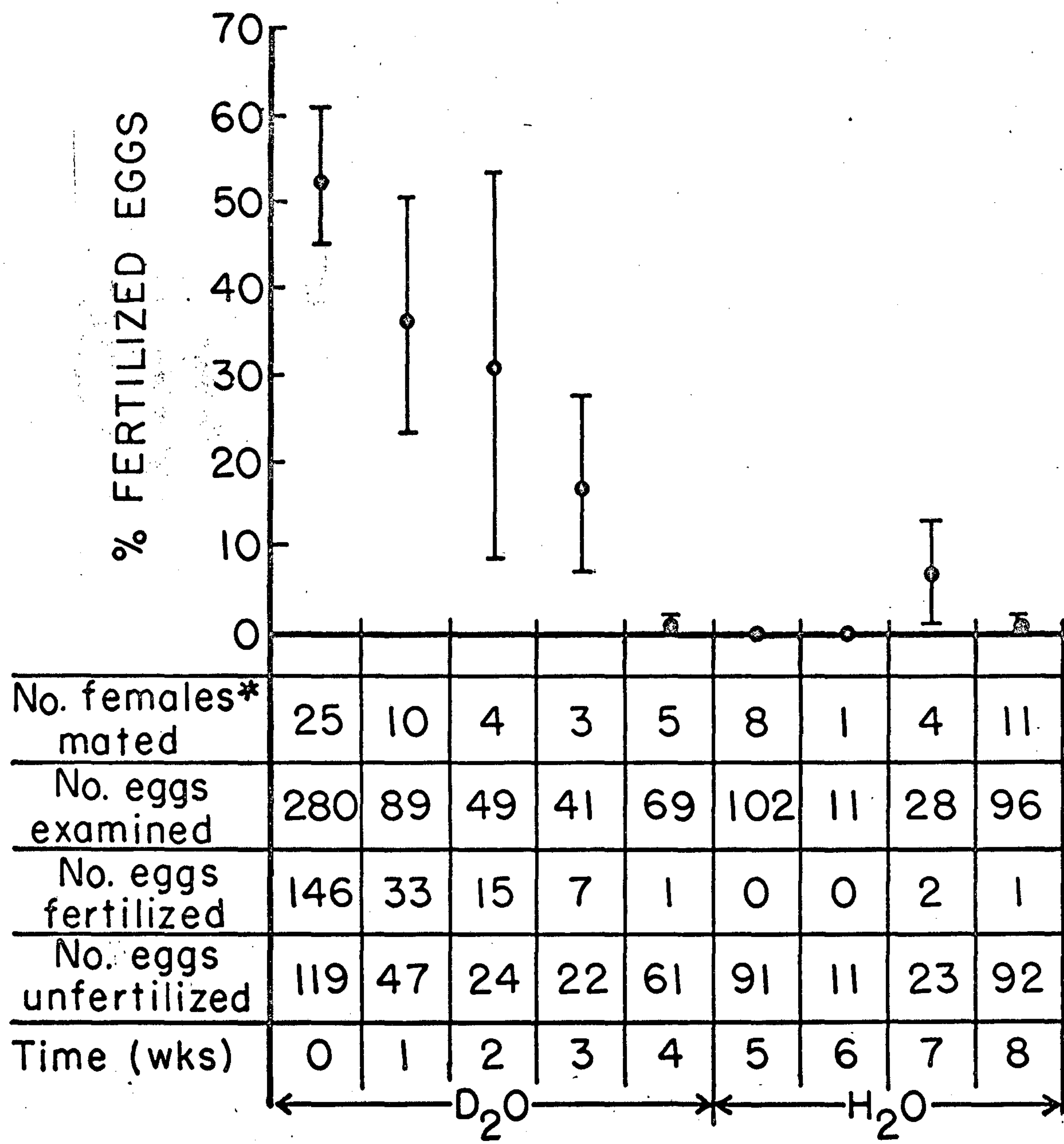
The effect of D_2O on the number and size of litters obtained from females mated to treated males

# females mated	28*	8	5	6	3	8	2	7	8
# females littered	25	8	5	1	0	0	0	0	2
# viable offspring	206	52	18	2	0	0	0	0	7
time (weeks)	0	1	2	3	4	5	6	7	8
	←	D_2O			→	←	H_2O		→

*The data from females mated to control males was pooled with that from females mated to treated males at time zero.

* The data from females mated to control males were used in the analysis of variance calculations. However, since the sample size for each week was small, the data have been pooled with that from the treated males at zero tim.

Fig. 1. The effect of D_2O on the number of fertilized eggs obtained from females mated to treated males. The numbers of fertilized eggs as a percent of the total eggs examined, with the standard error of the mean, is presented; also a tabulation of total eggs, fertilized and unfertilized eggs. Those eggs scored as degenerating or undiagnosible are not included in the tabulation.



No. females* mated	25	10	4	3	5	8	1	4	11
No. eggs examined	280	89	49	41	69	102	11	28	96
No. eggs fertilized	146	33	15	7	1	0	0	2	1
No. eggs unfertilized	119	47	24	22	61	91	11	23	92
Time (wks)	0	1	2	3	4	5	6	7	8

Fig. 1