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Ann M. Hughes and Melvin Calvin

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ABSTRACT

Sterility in C₅₇ and Swiss mice has been produced by substituting D₂O for a part of the drinking water. The effective range lies between 5% and 30% D₂O. It appears that the effect is greater in C₅₇ males than females, and that the size and viability of the litter is affected.

STERILITY PRODUCED IN MICE BY DEUTERIUM OXIDE

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The present availability of D_2O at a reasonable price has stimulated an increased investigation of its physiological effects. The inhibition of ascites tumor growth and of algal reproduction has recently been reported by this laboratory^{1,2} and others.³

We have demonstrated the production of sterility in mice by the substitution of D_2O for a part of the drinking water. In the first experiment, six female and six male C_{57} mice that had been maintained on 30% D_2O in the drinking water for 4 weeks were mated.[†] The animals were housed 3 females and 3 males to a cage. D_2O administration was continued for 10 weeks. Since there were no pregnancies at the end of this time, the D_2O was discontinued. At the end of another 8 weeks, when there still were no pregnancies, three of the treated females were mated with three normal males; three of the treated males were mated with three normal females. Although the mating of D_2O -treated females with normal males resulted in litters at the end of 3 weeks, all offspring died within 24 hours and two of the mothers died. The mating of D_2O -treated males with normal females did not produce offspring until the end of 13 weeks, at which time one female littered. From the 3 D_2O -treated males and 3 D_2O -treated females remaining together, one female littered in 4 additional weeks, one in 10 additional weeks. This experiment is graphically represented in Fig. 1.

In the second experiment, both C_{57} and Swiss mice were used. Five mice of each sex of each strain were maintained for 4 weeks on either 30%, 20%, or 5% D_2O in the drinking water. At the end of this treatment period, D_2O administration was discontinued and each mouse was individually mated with a normal mouse of the same strain. At the same time, six pairs of normal mice of each strain were mated. Only those litters born during a 30-day period after the beginning of the mating are included in the results.

[‡]All D_2O percentages are expressed as volume percent, approximately equal to atoms percent.

[†]As used in this paper, the term mated indicates that males and females were housed together continuously.

¹Hughes, Tolbert, Lonberg, and Calvin, *Biochim. et Biophys. Acta* (in press).

²Holm-Hansen, Moses, and Calvin; *Biochim. et Biophys. Acta* (in press).

³A. J. Finkel and D. Czajka; *Proc. Am. Assoc. Cancer Research*, 2, 201 (1957).

Our data, summarized in Table I, indicate that D_2O causes a sterility in both C_{57} and Swiss mice, and that the effect is greater in the C_{57} mice. It also appears that there is a greater effect in C_{57} males than in females, and that the size and viability of the litter is affected. Further experiments are in progress to verify these latter observations and to investigate some metabolic effects of D_2O in mice.

The failure of Hansen and Wulfert⁴ to observe sterility in mice as a result of D_2O is probably due to the low D_2O concentration they used by them.

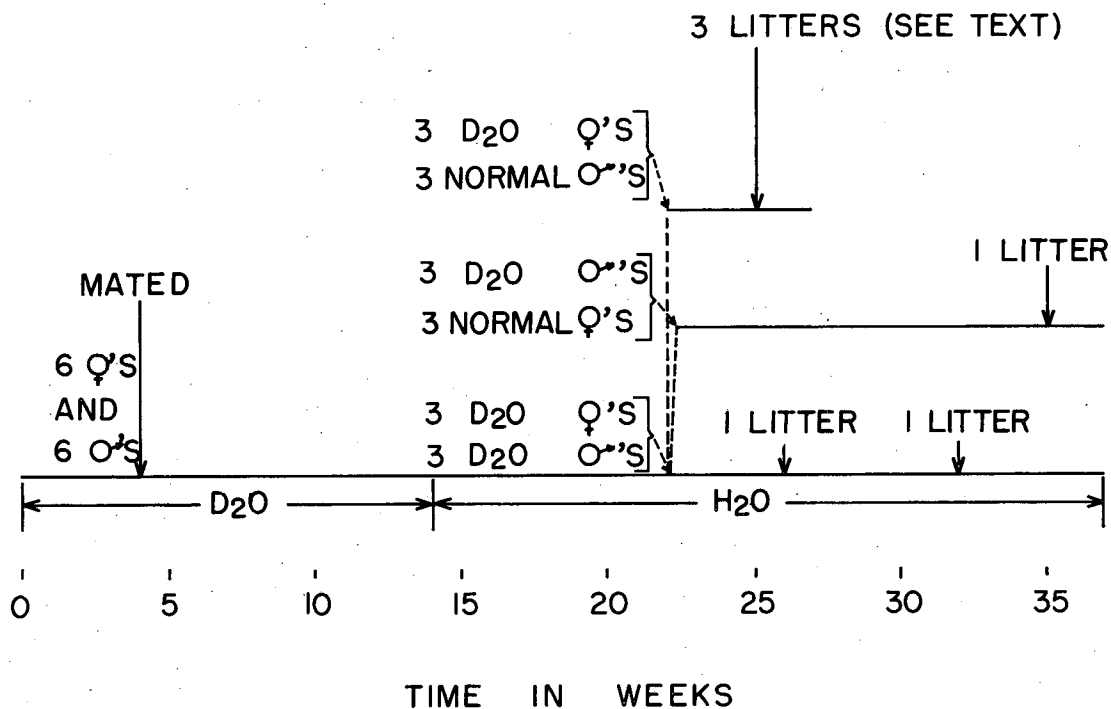
This work was done under the auspices of the U.S. Atomic Energy Commission.

⁴K. Hansen and K. Wulfert, Arch. exptl. Pathol., Pharmakol. Naunyn-Schmiedelberg's 190, 671 (1938).

Table I

The effect of D_2O on the fertility of C_{57} or Swiss Mice, presented as the number of surviving offspring per mated pair.

Sex treated with D_2O	D_2O conc. in drinking water (%)	Number surviving (offspring per mated pair)	
		C_{57}	Swiss
Male	5	1.2	7.2
Female	5	2.4	7.6
Male	20	1.0	7.4
Female	20	2.5	5.8
Male	30	0.0	2.0
Female	30	2.2	3.2
Controls	0	3.3	8.5



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Fig. 1. The production of sterility in C₅₇ mice by the administration of 30% D₂O in the drinking water. Horizontal arrows indicate length of time animals were given D₂O or H₂O. Vertical arrows indicate time of mating or littering. Dotted lines indicate time of cross-mating the treated with the normal animals, as described in the test.