Effect of heavy water on hepatic lipogenesis in mice

Since the discovery of deuterium the effects of this isotope on many diverse biological systems have been studied[1]. In general it has been shown that deuterium in high concentrations is toxic to all organisms, the degree of toxicity being greater in more highly organized organisms. BARBOUR[2-4] carried out a number of studies on the effects of D_2O ingestion on mice and concluded that when the body water is only 0.2 saturated with D_2O the mice survive but show elevated metabolism. KATZ *et al.*[5] have demonstrated that mice can be maintained on 25 % D_2O in their drinking water for periods up to a year with no adverse effects. Our own observations that tissue-culture cells maintained in 25 % D_2O exhibit lipid-containing inclusions[6,7] prompted an investigation into the effects of D_2O feeding on hepatic lipogenesis, which is the basis of this report.

Swiss mice were maintained on water containing 25 % D_2O, with *ad libitum* access to food and water. Every week, for three consecutive weeks, six D_2O mice and six controls were sacrificed and liver homogenates were prepared for studies of hepatic lipogenesis from sodium [2-^{14}C]acetate, using the technique described by RABINOWITZ AND GURIN[8]. Individual incubations were carried out for each liver. Cholesterol and fatty acids were isolated by previously described methods[9]. The results are presented in Table I.

TABLE I

EFFECT OF D_2O FEEDING (25 %) ON HEPATIC FAT SYNTHESIS IN MICE

Weeks	*Diet*	*Cholesterolgenesis*		*Fatty acid synthesis*	
		Counts/min/mg C × 10^3	*% Reduction*	*Counts/min/mg C* × 10^3	*% Reduction*
1	H_2O	1.57 ± 0.16*		9.50 ± 0.99	
	D_2O	1.46 ± 0.18	8	4.92 ± 0.69	49
				(0.01 < P < 0.001)	
2	H_2O	1.58 ± 0.14		3.26 ± 0.54	
	D_2O	1.12 ± 0.16	30	3.31 ± 0.17	—
		(P = 0.05)			
3	H_2O	1.10 ± 0.21		5.55 ± 0.17	
	D_2O	0.69 ± 0.10	38	3.15 ± 0.22	44
		(P = 0.10)		(P < 0.001)	

* Standard error of the mean.

It is evident that in D_2O-fed mice there is a general reduction in hepatic lipogenesis which becomes more pronounced with continuing D_2O ingestion. Histological examination of livers from D_2O-fed mice (carried out by Dr. V. DEFENDI) showed an increase in sudanophilic material over the controls and a progressive increase in sudanophilia as D_2O imbibition continues. Accumulation of liver cholesterol has been shown to inhibit cholesterogenesis[10,11] and our results might be explained on this basis.

To investigate the effect of D_2O concentration in the liver homogenate parallel experiments were carried out in which the buffer solution was taken to dryness under

reduced pressure and then reconstituted in water or in D_2O (> 99.5 % D_2O). The pH of both solutions was 7. In the homogenates prepared with heavy water the D_2O concentration was about 75 %. In each experiment six incubations were carried out with each buffer solution. The results are summarized in Table II.

In homogenates containing D_2O there was a considerable enhancement of lipogenesis. We may assume that the lipid accumulation in the livers of the D_2O-fed

TABLE II

EFFECT OF D_2O INCUBATION ON HEPATIC FAT SYNTHESIS IN MICE

Expt.	*Medium*	*Cholesterolgenesis*		*Fatty acid synthesis*	
		Counts/min/mg C × 10^4	% *increase*	*Counts/min/mg C* × 10^4	% *increase*
1	H_2O	1.00 ± 0.43*		2.84 ± 0.09	
	D_2O	1.31 ± 0.05	31	3.24 ± 0.05	14
		($P < 0.001$)		($0.01 > P > 0.001$)	
2	H_2O	1.29 ± 0.04		2.55 ± 0.14	
	D_2O	2.12 ± 0.10	65	3.69 ± 0.09	45
		($P < 0.001$)		($P < 0.001$)	

* Standard error of the mean.

mice was due to accelerated lipogenesis during the early days of feeding. The influence of D_2O on various enzyme systems was studied by a number of early investigators[1] with effects ranging from enhancement of activity to inhibition. The mechanisms underlying our observations await further study.

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