Changes in Renal Function in Rats Drinking Heavy Water.* (23871)

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Among changes observed in rats drinking heavy water were increases in blood levels of NPN and urea(1). These increases, suggestive of impaired renal function, were apparent when about 15% of body water was replaced by D_2O; at 30% replacement, the lethal level, concentration of urea in blood was 4 times the normal value. Dybing *et al.*(2) reported that livers and spleens of mice treated with D_2O showed degenerative changes, but that kidneys were essentially normal. In rats, however, evidence of renal tubular damage has been observed histologically(1). This paper is concerned with the effect of heavy water on glomerular filtration and renal plasma flow in rats.

Methods. The rats used were Sprague-Dawley females, about 7 months old and weighing 250-280 g. They were given 50 mole % D_2O as drinking water, and received Rockland rat diet *ad libitum*. The concentration of D_2O in plasma was estimated by the method of Katz *et al.*(1). Studies of renal function were carried out essentially according to the method of Smith and Boss(3). Rats were given two 10-ml doses of water by stomach tube 50 minutes apart. The D_2O content of the water was adjusted to that of plasma. After each dose of water, 250 mg/kg of creatinine as a 5% solution, and 150 mg/kg of sodium *p*-aminohippurate (PAH) as a 1.5% solution in 2% sodium sulfate were injected subcutaneously. Immediately after second injection the bladders were emptied by suprapubic compression. The rats were then placed in metabolism cages and urine collected for 50 minutes. At this time, blood was taken from each rat by heart puncture, and plasma and urine were analyzed for creatinine and PAH. Volume of urine collected was recorded, although the values reported in Table I are consistently low because of incomplete drainage from the collection funnels. The analyses of course were carried out on urine combined with washings of the funnels. Measurements of renal function were made on 3 occasions before D_2O was given, and 6 times during the 38-day period when rats were drinking D_2O. After 3 of the rats died, the remainder were given H_2O to drink, and the studies were repeated 1 and 5 days later.

Results. Table I shows changes in urine output, creatinine clearance (C_{Cr}), and PAH clearance (C_{PAH}) in rats drinking D_2O. Within 9 days all had decreased significantly, and by the 36th day all were only about 40% of control values. Recovery of kidney function following restoration of H_2O was remarkably fast: even at 1 day after removal from D_2O a marked improvement in kidney function was apparent, and after 5 days all were actually slightly above normal values.

In another less extensive experiment we had previously observed similar changes in respect to C_{Cr}: at 28% replacement of body water by D_2O, glomerular filtration was reduced to 45% of control level; 8 days after restoration to H_2O, recovery was virtually complete (95%).

It is clear from Table I that the rats immediately ceased to gain weight when given D_2O, although extensive weight loss was observed only in 2 animals, both of which died. Deaths occurred on 33rd, 36th, 38th, and 40th days after beginning of experiment; the last death occurred after replacement of D_2O with H_2O. The other 4 animals apparently recovered completely. At no time during the study was a difference evident between rats that subsequently died and those that ultimately survived.

Discussion. The fact that both glomerular filtration (C_{Cr}) and renal plasma flow (C_{PAH}) decreased at approximately the same rate and to about the same extent, while D_2O concentration in the body was rising toward 30 mole %, suggests that there was no specific injury to the kidney *per se*. The rapidity with which animals recovered when transferred from D_2O to H_2O is also consistent with the idea that

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TABLE I. Effect of D_2O on Kidney Function in Rats.

Days on D_2O	Plasma D_2O (mole %)	No. rats	Body wt (g)	ml/min./100 cm² surface area: Urine output Avg	Urine output S.E.	C_{Cr} Avg	C_{Cr} S.E.	C_{PAH} Avg	C_{PAH} S.E.
−50		7	246	.033	.003	.521	.050	2.57	.20
−43		8	251	.027	.003	.616	.025	2.22	.06
− 2		8	266	.031	.002	.521	.027	2.54	.20
Avg pre-D_2O		23	—	.030	.002	.551	.022	2.43	.10
3	9.7	8	261	.022	.002	.592	.036	2.50	.17
9	18.2	8	265	.015	.001	.396	.028	1.53	.19
15	20.6	8	263	.014	.001	.372	.037	1.71	.17
22	23.2	8	260	.017	.002	.283	.045	1.25	.11
29	26.0	8	258	.017	.003	.249	.039	1.52	.23
36	28.3	6	249	.013	.002	.228	.017	.92	.21
After H_2O									
1	19.8	5	254	.026	.004	.434	.039	1.42	.24
5	<5	4	250	.032	.005	.628	.012	2.60	.29

the lesions observed microscopically may be of no significance insofar as the survival of the animal is concerned. It is also relevant to point out that none of 6 enzyme systems in the kidney, including the oxidative phosphorylation system, was significantly altered, even in moribund animals with 30 to 32% of their body water replaced by D_2O(1).

The elevated concentrations of urea and NPN and the decreased concentrations of plasma protein and glucose in blood of rats receiving D_2O(1) are strongly suggestive of adrenal insufficiency, since these changes were *quantitatively* similar to those seen in adrenalectomized rats(4). It has been shown in several species that reduction of renal function occurs after bilateral adrenalectomy (see Smith(5) for review).

Thus it seems likely that one of the reasons for the toxicity of D_2O is a disturbance in adrenal function. In agreement with this supposition are the observations that hypophysectomized rats, with atrophic adrenal glands, show toxic effects at much lower levels of D_2O, and that in normal rats the adrenals increase in size by as much as 50%(1). Whether adrenal hypertrophy is actually accompanied by an increase in hormonal production has not been established. The D_2O-treated rat may have a higher requirement for adrenal hormones than can be met even by a hyperplastic cortex; the requirement may be unaltered but production of adrenal hormones may be inhibited in deuterated tissues; or metabolic processes of the rat may be so altered by deuteration that no additional amount of adrenal hormones would permit the animal to adapt to the altered internal environment produced by D_2O.

Summary. Glomerular filtration and renal plasma flow were decreased to about 40% of normal in rats drinking 50 mole % D_2O. Upon transfer from D_2O to H_2O, renal function rapidly returned to normal. It is probable that the changes in renal function do not represent a direct toxic action of D_2O on the kidney, but are perhaps the result of disturbed adrenal function.

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