Toxicity of Tritium Oxide to Mice. (19310)

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Tritium (H^3) is, in common with carbon-14, a radioisotope that may be used to label a large variety of physiologically important compounds. The soft nature of its β ray (mean energy about 5.7 kev) suggests interesting possibilities in the fields of radiobiology and therapeutics that might be achieved by selective localization in living structures. The biological effectiveness of this soft β ray is unknown, but the structures and functions sensitive to density of ionization appear to be predominant in determining radiation toxicity to the higher animals(1), so that direct experimental evidence regarding the biological effectiveness of tritium is important. Because of the low solubility of tritium or hydrogen gas in body fluids, it may be assumed that tritium water would be a considerably greater health hazard. These experiments were therefore done to establish the lethal dose of tritium water when injected intraperitoneally in physiological saline solution into CF No. 1 mice. Circumstances of the experimental conditions do not enable us to make rigid comparison of relative biological effectiveness as against better studied radiations, but do indicate clearly that no serious or unexpected correction factor needs to be introduced in calculating short-term tritium water toxicity on physical grounds.

Methods. Young female CF No. 1 mice, whose radiotoxic responses have been widely established in this laboratory, were injected intraperitoneally with 0.85% NaCl solution containing various amounts of tritium water diluted with sterile distilled water. Injection volumes ranged between 0.2 and 0.5 ml, and care was taken that none of the injection solutions escaped after removal of the needle. The mice were given water and Derwood checkers *ad libitum* and the intakes were measured daily. In a pilot experiment dosages of H_2^3O between 3.4 and 226 mc were given to mice weighing between 15 and 18 g; a subsequent experiment utilized mice weighing 20 $\pm$ 2.5 g, given dosages between 10 and 30 mc, bracketing the acute lethal range. The mortality results are shown in Table I.

Results. The tabulated results indicate a 30-day LD_{50} of about 1 mc per g body weight. Autopsy findings and hematologic observations indicate that deaths at and above this level were due to acute radiation disease.

Dosage calculations in physical terms would require serial analyses of body water specific activity and of uptake of tritium by organic compounds. but can be approximated by measurements of food and water intake. The

TABLE I. Mortality in CF #1 Mice Following Injection of H_2^3O.

Total dose, mc	No. of mice	Mice dying in 30 days — No.	Mice dying in 30 days — %	Median time to death
		Exp. T 1: mean wt of mice 16.5 g		
226	2	2	100	90 hr
113	2	2	100	96
34	4	4	100	7.5 days
11.3	4	0	0	
3.4	4	1	25	(at 13 days; probably not radiation death)
		Exp. T 2: mean wt of mice 20 g		
34	4	4	100	12 days
28.3	6	6	100	12
22.6	5	3	60	16
17	6	1	17	12
11.3	6	1	17	16
Controls	7	0	0	

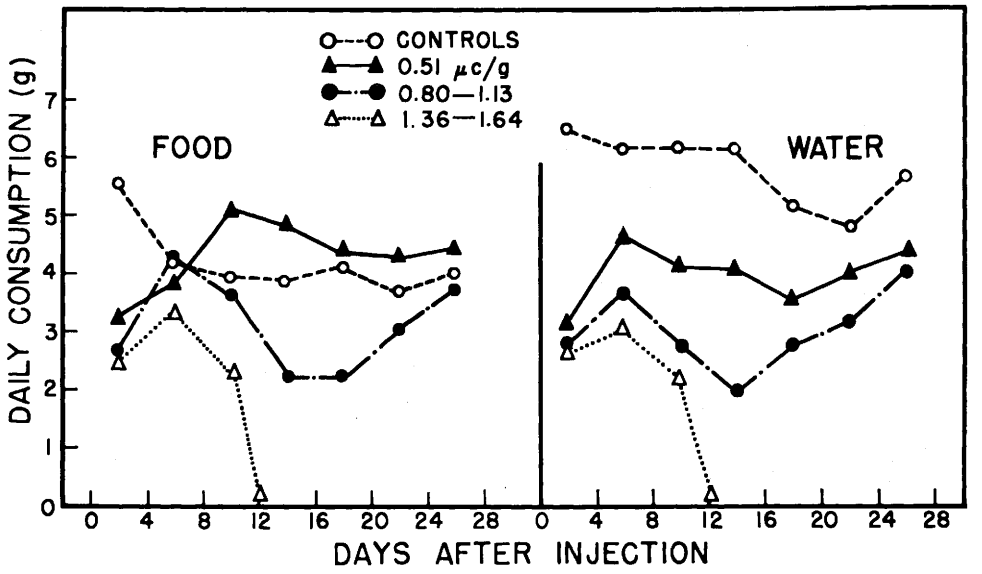

FIG. 1. Mean daily consumption of water and food by control and experimental mice divided into dosage groups as shown in the insert.

calculated total water intake (including metabolic water from food) in the mice receiving 20 and 15 mc in the second experiment indicated a rather constant water turnover for the first 4 days of 25% of the body weight per day, and for the second 4-day period this rose to about 42% per day. In Fig. 1 is shown the mean daily consumption of water and food in the various groups. The data indicate that treatment reduces the water intake more than the food intake, and that in the median lethal range food and water consumption are reduced most strikingly at the end of the second week. Assuming the body to be 75% water and that tritium oxide is excreted at the same rate as water, and disregarding the retarding effect of metabolic turnover of hydrogen and tritium, we have calculated the integrated total-body dosage over various periods of time at the several injection levels. These calculations appear in Table II. The 30-day LD_{50} for 250 kv X ray in these mice is about 575 r. Although no figures are available for estimating the lethal dose of X or γ rays administered with a time pattern such as this, it may be concluded that no remarkable factor of biological effectiveness exists for tritium β rays in relation to these better known radiations.

It is worth while remarking that dosages of tritium water in the lethal range tend to perpetuate themselves in the body due to the decreased food and water intake characteristic of the acute radiation response. This is illustrated by the relative rates of decrease in calculated dosage appearing in Table II. It should also be recognized that the relatively slower water turnover of the human being would result in a greater retention of the isotope and an increased toxicity, perhaps by a factor of 3 or 4, unless appropriate therapeutic measures were used.

Summary. The 30-day median lethal dose

TABLE II. Estimated Total-Body Dosage from Single Inj. of H_2^3O in CF #1 Female Mice.

Dosages, mc	mc/g	Initial rep/day	Dosages in rep 1-4 days	5-8 days	9-12 days	Total 1-12 days
11.3	.51	177	360	62	7	429
17	.80	264	605	155	18	778
22.6	1.13	353	812	206	24	1042
28.3	1.36	442	904	254	63	1221
34	1.64	530	1199	326	107	1632

of H_2^3O for CF No. 1 female mice is about 1 mc per g body weight when given in a single injection. The estimated radiation dosage is about 800 rep in the first 3 days and about 1000 rep in the first 12 days after injection. It is concluded that no unexpected factor of biological effectiveness exists for tritium β rays in relation to X or γ rays.

1. Zirkle, R. E., *Am. J. Roentgenol. Radium Ther.*, 1950, v63, 170.

Received December 10, 1951. P.S.E.B.M., 1952, v79.

Cation Exchange Properties of Alginic Acid. (19311)

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The ability of cation exchange resins to withdraw sodium from the body(1) suggested the investigation of cation adsorbing properties of various naturally occurring substances. This report summarizes the results of *in vitro* experiments conducted to determine the cation adsorbing capacity and ion selectivity of alginic acid. For comparison, similar studies were made with a carboxylic type cation exchange resin Natrinil.*

Alginic acid is obtained from the giant kelp (*Macrocystis pyrifera*). It is a polymeric anhydro-β-D-mannuronic acid chemically related to cellulose and pectic acid. Alginic acid possesses one carboxy group for each anhydromannuronic acid residue, and this free carboxy group is responsible for its ability to form water soluble gels with alkali metal ions. Alginic acid has a theoretical cation combining capacity of 5.7 meq/g.

The sodium and potassium salts of alginic acid are readily soluble in water. For this reason, separation of uncombined cations from the soluble alginate salts cannot be made by the use of the ordinary filtration or centrifuging technics generally employed with cation exchange resins whose alkali metal salts are insoluble. Dialysis of the mixture through a suitable semipermeable membrane such as cellophane provides a satisfactory method of effecting the separation of the diffusible uncombined cations from the adsorbed cations.

* Natrinil is the trade mark of the National Drug Co. brand carboxylic type cationic exchange resin. In all experiments reported here, the acid form of this resin was employed.

Determinations of sodium, potassium and calcium were made using a Perkin-Elmer Model 52-C Flame Photometer. In determining calcium the characteristic calcium oxide band spectrum was employed. In each procedure described, suitable control experiments were conducted to establish the validity of the method under the conditions utilized.

1. *Adsorption of Na^+ from sodium chloride solutions.* The weighed sample of alginic acid was allowed to equilibrate with a sodium chloride solution by shaking at room temperature for 3 hours. The mixture was then dialysed against 3 successive portions of 1 liter of water for a total period of about 40 hours. Control experiments indicated that these dialysis conditions permitted practically quantitative diffusion of the uncombined cations with negligible diffusion of alkali metal alginates. Sodium determinations were made on the supernatant liquid obtained by centrifuging the dialysis bag contents. Curves 3 and 4 of Fig. 1 show the amount of Na^+ adsorbed by 1 g alginic acid or 1 g Natrinil from solutions containing varying amounts of Na^+. The total volume of solution in each instance was 40 ml. Both alginic acid and Natrinil were poor sodium adsorbents in unbuffered sodium chloride solutions, with alginic acid more effective than the synthetic resin. The affinity of each of the adsorbents for sodium was not appreciably influenced by the ion concentration. The sodium adsorption in unbuffered solutions was accompanied by a marked increase in hydrogen ion concentration. Liberation of hydrogen ions by 1 g