Antioxidants and Survival Time of Mice Exposed to Multiple Sublethal Doses of X-Irradiation.* (25804)

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It has been suggested that the deleterious effects of ionizing radiation in animals are due, at least in part, to excessive formation of peroxides(1,2). If such were the case, preventing or minimizing peroxide formation following radiation might be expected to have a protective effect. Attempts to counteract radiation injury by administering tocopherol, a naturally occurring antioxidant, however, have been unsuccessful. Furth *et al.*(3) administered alpha-tocopherol in oil to rats prior to irradiation without beneficially improving survival. Haley *et al.*(4) found that intramuscular injections of a water-soluble vitamin E to mice immediately prior to x-irradiation were similarly without protective effect. On the contrary large doses of Vit. E significantly decreased survival following exposure to a lethal dose of total body x-irradiation in mice (4). Since antioxidants may differ in respect to their concentration and distribution in tissues as well as their capacity to inhibit peroxide formation *in vivo,* the present investigation was undertaken to obtain further data on effects of antioxidants of diverse structure and activity on length of survival following exposure to multiple sublethal doses of total body x-irradiation in the mouse.

Procedure. The basal ration employed in the present experiment consisted of cerelose, 59%; casein,† 24%; cottonseed oil, 10%; salt mixture,‡ 5%; cellulose,§ 2%; and the following vitamins per kg of diet: thiamine hydrochloride, 10 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 10 mg; calcium pantothenate, 60 mg; nicotinic acid, 100 mg; ascorbic acid, 200 mg; biotin, 4 mg; folic acid, 10 mg; para-aminobenzoic acid, 400 mg; inositol, 800 mg; Vit. B_{12}, 150 μg; 2-methyl-1, 4-naphthoquinone, 5 mg; choline chloride, 2 g; Vit. A, 5000 U.S.P. units; Vit. D_2, 500 U.S.P. units; and alpha-tocopherol acetate, 100 mg. The vitamins were added in place of equal amount of cerelose. Male mice of Webster strain were selected at 11 to 14 g in body weight and were divided into comparable groups consisting of 30 animals per group. These were placed in metal cages with raised screen bottoms (5 animals per cage) and fed either basal ration or basal ration plus the supplements listed in Table I. Supplements were added to basal ration in place of an equal amount of cerelose. Food and water were provided *ad libitum.* The animals were fed daily and all food not consumed 24 hours after feeding was discarded. After 6 weeks of feeding, all mice received an exposure of 200 r total body x-irradiation which was repeated once weekly until a total of 1600 r (8 exposures) had been administered. Animals were continued on their respective diets for 126 days after the first x-ray exposure or until death, whichever occurred sooner. All mice that died before the 4th x-ray exposure were not included in tabulation of data. This procedure was employed to minimize variations due to early deaths or atypical responses on the part of individual mice. Data were obtained as to the day after first x-ray exposure when 50% of the animals in each group were dead and average length of survival on each of the diets employed. The following radiation factors were employed: GE Model Maximar 250; 250 kv; 15 ma; 0.5 mm Cu and 1 mm Al filters plus a Cu parabolic filter;‖ HVL, 2.15 mm Cu; target distance to top of box, 82 cm; and dose rate, 15.6 r per

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† Vitamin-free Test Casein, General Biochemicals, Chagrin Falls, O.

‡ Wesson Modification of Osborne-Mendel Salt Mixture, General Biochemicals, Chagrin Falls, O.

§ Solka Floc, Brown and Co., Boston, Mass.

‖ A nonuniform filter which produces a flat isodose surface of x-ray intensity constructed by the method of Greenfield and Hand(5). Center of filter was 1.7 mm Cu; the edge, 0.5 mm Cu.

TABLE I. Effects of Antioxidants on Survival Time of Mice Exposed to Multiple Sublethal Doses of Total Body X-irradiation.*†

Dietary group	No. of animals	Avg body wt at time of 1st x-irrad. (g)	Day after 1st x-irrad. when 50% of animals were dead	No. surviving‡	Avg length of survival after 1st x-irrad. (days)§
Basal ration	29	29.2	64	2	67.6 ± 5.3‖
Idem + following supplements:					
.25% mixed tocopherols	28	29.4	63	1	65.8 ± 4.6
.25% Santoquin	29	25.4	60	3	66.4 ± 4.5
.25% DPPD	29	28.7	66	5	75.4 ± 5.8
.5% "	28	28.3	68	6	75.3 ± 6.4
.25% propyl gallate	28	28.7	82	7	83.9 ± 6.8
.5% " "	28	28.0	93	9	85.5 ± 7.0
.25% DBH	26	27.4	96	8	89.2 ± 6.7
.5% "	26	25.5	82	3	84.6 ± 5.5
.25% BHT	24	28.2	95	9	93.5 ± 6.6
.5% "	28	26.7	>126	16	96.9 ± 7.0

* Each group initially consisted of 30 mice. Animals that died before 4th x-irradiation were not included in Table.

† Test supplements were obtained from following sources: Mixed tocopherols concentrate N.F., Type 4-50 (each g contains 500 mg of mixed tocopherols, equivalent to 372.5 international units of vit. E), Distillation Products Industries, Rochester, N. Y.; Santoquin, Monsanto Chemical Co., St. Louis, Mo.; DPPD, B. F. Goodrich Chemical Co., Cleveland, O.; propyl gallate, Heyden Newport Chemical Co., N. Y.; DBH, Distillation Products Industries, Rochester, N. Y.; and BHT, Nopco Chemical Co., Harrison, N. J.

‡ Experiment terminated 126 days after 1st x-irradiation.

§ Data calculated on basis of avg survival time of 126 days for animals alive at termination of experiment.

‖ Including stand. error of mean calculated as follows: $\sqrt{\frac{x^2-\frac{(\Sigma x)^2}{n}}{n-1}} / \sqrt{n}$, where "x" = length of survival after 1st x-irradiation and "n" is No. of observations.

minute (measured in air, at top of box). The animals to be irradiated were placed in a wooden box divided into 60 equal compartments 1¼″ wide, 3″ long, and 1½″ deep. The partitions and top were made of ⅛″ cellulose acetate sheetings; and top and bottom of each compartment were perforated with holes for purposes of ventilation. The container was rotated slowly on an electrically driven turntable to insure equivalent irradiation.

Results. Findings are summarized in Table I. Significant differences occurred between the various groups in respect to survival following exposure to multiple sublethal doses of total body x-irradiation. Whereas mixed tocopherols and Santoquin (6-ethoxy-2, 2,4-trimethyl-1, 2-dihydroquinoline) at a 0.25% level in the diet¶ and DPPD (N, N′-diphenyl-p-phenylenediamine) at levels of 0.25% or 0.5% of the diet had little if any effect on length of survival, the day 50% mortality was attained or number of animals surviving, propyl gallate, DBH (2,5-di-tert-butyl-hydroquinone) and BHT (butylated hydroxy toluene) at levels of 0.25% or 0.5% of the diet all resulted in increased survival compared to that of animals fed the basal ration. Findings were particularly significant in the case of BHT.

Discussion. Present findings indicate that substances with antioxidant activity can significantly prolong survival following exposure

¶ An additional group was started, fed Santoquin at 0.5% level in the diet. Fifteen of 30 mice died during first 6 weeks of feeding and survivors averaged only 18.1 g in body weight on day of 1st x-irradiation. In view of toxicity of Santoquin at this level of feeding, these animals were discarded and not included in the radiation experiment.

to multiple sublethal doses of total body x-irradiation in the mouse. Not all substances with antioxidant activity, however, were active in this regard. Thus, whereas BHT and to a lesser extent DBH and propyl gallate prolonged survival under conditions of the present experiment, mixed tocopherols and Santoquin were inactive at the levels fed and DPPD exhibited only questionable activity. The protective effect of BHT, DBH and propyl gallate are consistent with the suggestion that the deleterious effects of ionizing radiation are due, at least in part, to peroxide formation(1, 2) and that antioxidants by preventing or minimizing peroxide formation exert a protective effect. The mixed tocopherols, which are naturally occurring antioxidants, were far less active, however, than the synthetic antioxidants BHT, DBH and propyl gallate, suggesting that the latter compounds might be more effective than tocopherols in reaching the site of peroxide action, or in their capacity to inhibit peroxide formation. If such were the case, it is possible that other antioxidants might be even more effective against radiation injury. The possibility has not been excluded, however, that the protective effect of BHT, DBH and propyl gallate is due to some mode of action other than their antioxidant activity.

Summary. Experiments were conducted to determine effects of antioxidants on survival time of mice exposed to multiple sublethal doses of total body x-irradiation. Mixed tocopherols and Santoquin at 0.25% level in the diet, and DPPD at levels of 0.25% or 0.5% of diet had little if any protective effect. Propyl gallate, DBH and BHT at levels of 0.25% or 0.5% of the diet increased survival over that on basal unsupplemented ration.

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Effect of Osmotic Diuresis on Renal Blood Flow.* (25805)

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The demonstration that osmotic diuresis has a protective effect against the tubular lesions following renal ischemia, first by Owen and associates(1), and later by Hatcher and his associates(2), arouses interest in what peculiar properties of osmotic diuresis might be responsible for this effect. Very little study has been devoted to effects of osmotic diuresis on renal function and hemodynamics. Earlier studies have been focused on the mechanisms by which osmotic diuresis was produced. This paper presents evidence that osmotic diuresis under certain conditions may affect renal blood flow.

Methods. Patients undergoing differential renal function studies in investigation of hypertension were used in this study. Only patients whose clearance studies revealed renal blood flow and glomerular filtration to be within normal limits were included. After ureteral catheterization and after obtaining a blood and urine blank specimen, priming and maintenance infusions of sodium paraaminohippurate and inulin were begun in accordance with previously described technics(3). Previously, patients had been hydrated by oral intake of at least 1000 cc of water before the experiment began. One hour was allowed for

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